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CRITICAL REMARKS CONCERNING THE VALIDITY OF THE MITSCHERLICH EFFECT LAW *)

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(Mit deutscher Zusammenfassung)

A few years ago Mitscherlich[3]) and Gericke[1]) published a verification of the validity of the Mitscherlich Effect Law based upon the results of more than 27000 field tests with graded amounts of P_2O_5, K_2O and N, which have been laid down in the pre-war years on the principal soil types of Germany, using the principal crops as test plants.

The Mitscherlich Law is represented by the following equation:

$$\log (A - y) = \log A - c(x + b),$$

meaning that the increase of the yield of any crop under the influence of increasing amounts of any growth factor x is proportional to the difference between a partial yield y obtained at any stage and a certain maximum possible yield A; c is a constant that depends on the nature of the growth factor and determines the slope of the yield curve. The quantity b represents the amount of x originally present in the soil.

The use of average results from large numbers of experimental fields has the advantage that the influence of accidental deviations and errors is to a large degree eliminated.

According to both these investigators the validity of the equation was confirmed. Consequently the relation between yield and the amounts of any fertilixer may be represented by a logarithmic curve which is identical for all crops under investigation. The constant c is approximately equal in all cases with the same nutrient. This result

*) W. Stollenwerk in a recent paper[5]) criticizing the work of Mitscherlich and Gericke but employing different methods attained approximately similar results to those of the present paper.

is considered by Gericke to be an average one, from which individual deviations occur. Mitscherlich's conclusion agrees with that contained in former publications and is very definite: "Das Wirkungsgesetz der Wachstumsfaktoren als solches besteht und ist richtig" *).

From the constancy of c it is deduced by Mitscherlich that the need for any plant nutrient of all crops is quite similar: "Alle unsere Kulturpflanzen reagieren hinsichtlich der Nährstoffausnutzung zur Ertragsbildung ganz in gleicher Weise! Es gibt so z.B. keine Kulturpflanze, die vielleicht die Bodenphosphorsäure besser aufschlösse als eine andere! Diese Ansicht gehört in das Reich der Fabeln".

It is no wonder that the results obtained by these well-known German investigators has evoked interest elsewhere.

Willcox [7]) has drawn them to the attention of English readers and emphasizes the outstanding importance of this work. He refrains however from any criticism of the methods applied by Mitscherlich and Gericke.

Since many of the present readers may not be well acquainted with the extensive work of Mitscherlich and the widespread criticisms of it (Rippel [4]), Meyer [2], de Vries [6]) a.o.), it seems worth while to criticize in detail the method of calculation and the interpretation of the results.

CALCULATION AND INTERPRETATION OF RESULTS

The procedure of the authors was to take all the series of tests with each of the different crops and to compute the average yields of the treated and the untreated plots. Accepting the values $c = 0.6$ for P_2O_5, $c = 0.4$ for K_2O and $c = 0.6$ for N which values were derived from previous investigations, the maximum value A was calculated from the differences between the average yields of the untreated and those of each of the treated plots.

This method of computation of A gave 4 different values of A, from which the average was calculated. This is statistically unwarranted. The yield of the untreated plot is used 4 times, viz. in each of the (4) equations with the yields of the other plots. Consequently a

*) There is a law governing the effect of growth factors which is exact.

possible error in the yield of the untreated plot has too heavy an influence on the value of A.

An improved method of calculation is given below.

It was supposed by Mitscherlich and Gericke that the averages of the plots from a large number of experiments treated in a similar way can be used for an evaluation of the validity of the law. A mathematical verification of this assumption can be given, assuming that c is really a constant for each crop.

Suppose that the following equation is valid for a certain crop in a certain case:

$$\log (A_i - Y_i) = \log A_i - c(x + b_i)$$

The index i indicates that the values concerned deal with the results of an experiment i of the series.

Then

$$\frac{A_i - Y_i}{A_i} = 10^{-c(x+bi)}$$

$$Y_i = A_i - A_i\, 10^{-c(x+bi)} = A_i - A_i\, 10^{(-cbi)}\, 10^{-cx}$$

Substitute for $A_i\, 10^{(-cbi)}$, which is a constant in any given experiment B_i, then the equation can be written as follows

$$Y_i = A_i - B_i\, 10^{-cx}$$

Obviously this equation will not describe exactly the results of a given experiment since the yield is subject to accidental fluctuations. Actually the equation must be written

$$y_i = A_i - B_i\, 10^{-cx} + \varepsilon_i$$

ε_i is the difference between the yield y_i found and the theoretical yield $Y_i = A_i - B_i\, 10^{-cx}$.

If the average yield $\bar{y}$ is computed for a distinct amount of fertilizer x from a large number of experiments, then

$$\bar{y} = \bar{A} - \bar{B}.10^{-cx} + \bar{\varepsilon}$$

from which it follows that the Mitscherlich Law for constant value of c is also valid for the averages.

It is further evident from this equation, that if y_i (or $\bar{y}$) is plotted against 10^{-cx} a straight line is obtained, from which an equation, since it is a regression line, can be computed in the usual way. This

computation also gives an estimate of s for the value of the standard error $\sigma = \sqrt{\Sigma(\varepsilon)^2/n}$ of the deviations from the regression line

$$s = \sqrt{\frac{\Sigma\,(\bar{y} - \overline{Y})^2}{n-2}} \simeq \sigma.$$

It is obvious too that the mean value of b indicating the amount of nutrient originally in the soil can be deduced from $\overline{B}$ by means of the formula

$$\overline{B} = \overline{A}\ 10^{-c\bar{b}} \quad \text{or} \quad \bar{b} = \frac{\log \overline{A} - \log \overline{B}}{c}.$$

The results have been examined by Gericke by computing the standard error of A determined in the manner critized above (p. 98). These errors appear small. Hence the conclusion is drawn that the Mitscherlich Law fits the data.

According to Mitscherlich the good agreement between the curve calculated from the results by means of the equation and the results really obtained is the proof of the validity of the Law. This judgment which is based upon a curve derived from the actual data is not very clear.

CRITICISM ON THE WAY OF JUDGMENT

In the opinion of the author it is not only important that the deviations should appear small but they should really be small in respect of the number of experiments involved. It must further be shown that the deviations found by applying the postulated values of c are smaller indeed than those found by applying other values of c.

The error in A is presented by Gericke in the form of the mean error which is an unusual statistic (Table I, a). We have calculated the standard error with the aid of the formula $s_A = \sqrt{\Sigma d^2/n - 1}$ *) using the data of Gericke. The percentage value of s: $100 s_A/\overline{A}$ is given (b). The standard error of y (as a percentage, $100 s/\bar{y}$) is also calculated from the deviations of the points from the curves calculated by Mitscherlich, using the formula $s = \sqrt{\Sigma d^2/n - 2}$ **) (as two constants A and b are adopted, it is necessary to divide by $n - 2$ (table I, c).

*) $d = A - \overline{A}$ **) $d = y - Y$.

TABLE I

Percentage errors found by different methods of computation									
	rye	oats	wheat	barley	sugar beet	mangold	potato	hay	clover
a. mean error found by Gericke	1.5	1.3	1.3	2.7	0.9	0.9	0.7	2.9	5.0
b. standard error of A $\frac{100\, s_A}{\bar{A}}$	2.0	1.8	2.1	3.8	1.3	1.4	0.9	3.4	7.8
c. standard error of y comp. from data of Mitscherlich $\frac{100\, s}{\bar{y}}$	2.5	2.2	2.6	4.7	1.6	1.7	1.1	4.2	9.6
d. $\frac{100\, s_A}{\bar{A}}$, own calcul.	1.0	1.0	1.4	1.4	—	0.8	0.4	2.0	2.5
e. $\frac{100\, s}{\bar{y}}$, own calcul.	1.3	1.3	1.4	1.8	—	0.9	0.5	2.7	3.3

As a result of the use of a curve less adapted to the points (resulting from the incorrect method of calculation mentioned above) the standard errors of A and y are decidedly higher than those deduced from our calculations (table I, *d* and *e*).

Should these standard errors (also the lower ones found by our calculation) really be considered negligibly small as has been asserted by Mitscherlich, Gericke and Willcox? This may be seriously doubted considering the number of experiments. If the factors phosphate, potash and nitrogen do not interact with other factors as postulated by Mitscherlich and if the effect factor c is equal in all experiments, then purely accidental errors would be reduced to the $1/\sqrt{n}$ part (that is to 1/41 of the original value in the case of potatoes, to 1/39 in the case of hay, to 1/35 in the case of rye etc. as these experiments have been performed 1642, 1535 and 1218 times respectively). This conclusion, assuming that c is a constant, follows from the justification of taking the average given above (p. 99). Consequently the standard errors $\sigma_i = \sqrt{\Sigma \varepsilon_i^2/n}$ of the single fields must have been extremely high. According to our calculations they would amount to 44% for rye, 40% for oats, 31% for wheat, 34% for barley, 20% for potatoes, 25% for mangolds, 104% for hay and 38% for clover. Thus it proves that no satisfactory confirmation of the Mitscherlich Law has been obtained.

COMPUTATION OF THE STANDARD ERROR USING OTHER VALUES OF c IN THE CASE OF PHOSPHATE EXPERIMENTS

Mitscherlich and Gericke have assumed a constant value of c. However, it ought to be the purpose of the investigation to trace in how far this assumption is permissable. The computations have therefore been repeated with different values of c. The values of c giving minimum values of s are determined by means of a calculation of A and b according to the method described above.

The phosphate experiments are most suited to this purpose as 5 plots dressed with different amounts of fertilizer (0, 30, 60, 90, 120 kg/ha P_2O_5) are available. The experiments with sugar beet where only 4 different amounts of phosphate were used are omitted, as the number of observations is too low for a reliable determination of a regression line. The potash experiments consisting of 4 dressed plots only are not considered here for the same reason. The nitrogen experiments are complicated by the occurrence of yield depressions.

A further objection must be made against the use of long term experiments for the present purpose, when the design of the experiments was similar in successive years. Phosphate manuring usually causes residual effects in following years. It follows that larger amounts of phosphate are responsible for the effects observed than the quantities actually applied in a certain year. Plotting the latter instead of the unknown larger amounts (added quantity + residual phosphate) against the yield results in a steeper slope of the curve which corresponds to a higher value of c. The exact values of c therefore are probably lower than those actually found.

The calculated values of c for minimum values of s are to be found in table II. With one exception these values of c are lower than 0.6 and are appreciably variable. The average value of c is 0.44 ± 0.09. The minimum values of s are considerably lower than the values of s found if $c = 0.6$. Again the percentage value of s using the best fitting value of c is lower than the corresponding value if $c = 0.6$ given in table I. The standard errors of the single fields presented in the last column of table II are generally lower than those found before. Nevertheless our conclusion based on the well-known variability in field trials that these errors are by far too high is also confirmed for varying values of c.

An exception must however be made in the case of potatoes. The

standard error of 2.3 corresponds to the order of the errors usually found in our own experiments performed with this crop. The value is even rather low. Thus it may be concluded that the Effect Law is satisfactorily confirmed for this crop, but that the value of c is 0.40.

The elimination of many secondary reactions in the case of potatoes as a result of the use of comparable plant material, number of plants etc., which are less readily realized for cereals (occurrence of tillering, fructification, maturation) may be suggested as reasons for the far better results obtained with potatoes. In addition it is possible that the value of c is more subject to variations with other crops.

TABLE II

Crop	c for min. s	minimum s	s if $c = 0.6$	$\frac{\text{min. } s}{\bar{y}}$ in %	$\frac{\text{min. } s}{\bar{y}} x\sqrt{n}$ in %
Rye	0.40	0,250	0.299	1.05	36.6
Oats	0.50	0.338	0.351	1.23	38.1
Wheat . . .	0.50	0.395	0.404	1.42	31.7
Barley . . .	0.60	0.468	0.468	1.77	33.7
Potatoes . .	0.40	0.144	1.270	0.06	2.3
Mangolds .	0.35	4.265	6.695	0.59	14.5
Hay	0.35	1.800	2.058	2,33	91.4
Clover. . .	< 0.10	—	2.744	—	—
mean . . .	0.44 ± 0.09				

The value of c found for mangolds is not particularly high. Yet this value is decidedly higher than which is usual for this crop. What has been said for potatoes is probably true of mangolds. However, the value of c is smaller again ($c = 0.35$) than that postulated by Mitscherlich.

No value of c has been determined for clover. A minimum value of s was not even reached with $c = 0.10$. It is biologically unlikely that c has a still lower value. Besides the results obtained with this crop are irregular.

Our conclusion is that the value of c in single experiments is subject to large variations (and this would also be true if experimental errors could have been avoided) or that considerable deviations of the logarithmic slope of the yield curve occur. According to our own experience the first possibility may be true in many cases.

COMPUTATION OF THE AMOUNT OF AVAILABLE PHOSPHATE IN THE SOIL

Accepting $c = 0.6$ for phosphate Gericke has calculated the value b (in quintals/ha P_2O_5) for various crops. These values vary between 0.82 for hay and 1.46 for sugar beets. We have found (table III) a variation between 0.82 (hay) and 1.31 (potatoes), and for arable crops only 1.01 — 1.31. It is however clear that this calculation has lost its theoretical foundation if the suggested constancy of c is not confirmed. Larger variations are found when using the correct values of c for minimum s (table III).

TABLE III

crop	b in the case of		c for s minimal
	$c = 0.6$	value of c if s_a = minimal	
Rye	1.01	1.11	0.40
Oats	1.05	1.21	0.50
Wheat	1.16	1.34	0.50
Barley	1.16	1.16	0.60
Mangold	1.31	1.83	0.35
Potato	1.22	1.78	0.40
Hay	0.82	1.19	0.35
Clover	0.89	—	< 0.10

It follows from table III that the available amount of phosphate varies rather significantly (for arable crops only 1.11 — 1.83). Of course there is no a priori objection to the creation of a conventional method of soil testing by means of the application of a postulated constant value of c for all crops. However, such a method (the Mitscherlich pot culture method) is not principally distinguished from any other conventional method of soil testing. It has no greater merit than other methods from a physiological point of view as has been claimed by Mitscherlich. This method can only be evaluated by means of field tests. It has no value in itself as a physiological foundation is absent.

It is clear from the foregoing that the conclusion of Gericke with regard to the different phosphate contents of the German soil types, on which the various crops were grown, are erroneous.

Acknowledgement. The author is much indebted to Dr E. F. Drion of the Statistics Department T.N.O. for valuable criticism, mathematical amendments and the performance of calculations.

SUMMARY

A criticism is made of the evaluation of the Mitscherlich Effect Law on the basis of more than 27000 field experiments with graded amounts of fertilizers by Mitscherlich and Gericke. Accidental errors are eliminated to a large degree by the use of large numbers of field tests. According to these investigators (also Willcox) a very good confirmation of the law has been obtained, and the discrepancies found should be small and without any real significance.

In contradiction to this view it is shown in the present paper that the discordance is important considering the large number of experiments. An exception should be made in the case of potatoes in which a confirmation of the Law was obtained. The principal cause of these discrepancies is probably the variability of the effect factor c of the fertilizer involved in single experiments.

The constancy of $c = 0.6$ as for phosphate suggested by Mitscherlich was not confirmed for various crops. Better fitting results were obtained with different values of c. These values varied in the case of phosphate between 0.35 for mangolds and 0.60 for barley with an average value for all crops of 0.44 ± 0.09.

It is probable that the values of c found are somewhat too high as a result of the residual effects involved in long term phosphate experiments.

The determination of the amount of phosphate b in the soil gave rather discrepant results. The method of soil testing adopted by Mitscherlich based upon the assumption of a constant value of c lacks a physiological foundation. Any advantage of this method over other conventional methods of soil testing is denied.

ZUSAMMENFASSUNG

Kritische Bemerkungen zur Gültigkeit des Mitscherlichschen Gesetzes der Wachstumsfaktoren

Es wird die Prüfung der Gültigkeit des Mitscherlichschen Gesetzes, wie diese anhand von 27000 Feldversuchen von Mitscherlich und Gericke durchgeführt wurde, kritisiert. Der Vorteil einer Benutzung größerer Zahlen von Versuchen ist daß zufällige Fehler eliminiert werden können. Nach der Meinung beider Förscher (auch Willcox) wurde eine recht befriedigende Bestätigung des Gesetzes erhalten. Die gefundenen Abweichungen würden klein und ohne wesentliche Bedeutung sein.

Im Gegensatz zu dieser Auffassung wird aber gezeigt, daß die Abweichungen unter Berücksichtigung der großen Zahl der Versuche beträchtlich sind (Tabelle II). Eine Ausnahme macht nur die Kartoffel, mit welcher eine Bestätigung des Gesetzes gefunden wurde. Die Hauptursache der Abweichungen ist wahrscheinlich die Inkonstanz des Wirkungsfaktors c in den verschiedenen Versuchen.

Die Konstanz von $c = 0.6$, wie diese Mitscherlich als erwiesen betrachtet, konnte für verschiedene Gewächse nicht gefunden werden. Genauere Angleichung der Daten wurde mit wechselnden Werten für c erhalten. Diese Werte lagen zwischen 0.35 für Futterrüben und 0.60 für Gerste, der mittlere Wert betrug 0.44 ± 0.09. Es ist aber wahrscheinlich daß die gefundenen Werte von c bei den mehrjährigen Feldversuchen infolge der Nachwirkung der Phosphatdüngung mehr oder weniger zu hoch sind.

Die Bestimmung der im Boden vorhandenen wirksamen Phosphorsäuremenge b ergab ziemlich auseinanderliegende Werte. Die Mitscherlichsche Methode der Bodenuntersuchung, welche sich auf die Annahme konstanter Wirkungsfaktoren stützt, entbehrt einer physiologischen Grundlage. Die Vorteile dieser gegenüber den chemischen Methoden werden verneint.

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NUTRITIONAL STUDIES ON FRUIT TREES

II. THE RELATION BETWEEN POTASSIUM, MAGNESIUM AND PHOSPHORUS IN APPLE LEAVES

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INTRODUCTION

Substantial evidence has been accumulated in the past suggesting that magnesium has a function as a carrier for phosphorus. This has already led to the use of superphosphate in conjunction with magnesium silicates. As far as fruit trees are concerned, however, there is little or no literature on the subject.

Lilleland who studied the phosphate nutrition of apples and peaches during the period 1936–42 [4] [5] [6] [7] does not mention any relationship with magnesium. He concluded that with improved growth, the phosphate content of the leaves decreases, thereby indicating a negative correlation between nitrogen and phosphate uptake.

Following studies on magnesium deficiency, we were able to compare the phosphorus contents of apple leaves sampled from different soils and found that samples with low potassium contents contained larger amounts of phosphorus than did normal ones. This was particularly noticeable when samples from a potassium deficient river clay were compared with samples from a marine loam, rich in potassium. Comparisons between samples from marine loams with a normal potassium content and those from loams with an abnormally high potassium content (resulting in symptoms of magnesium deficiency) did not, however, give the same result. It appears therefore, that the difference in potassium content has to be rather large before there is any significant difference in phosphate content.

METHODS

Rapid plant tissue test method

The normal Morgan extracting solution consisting of 10% sodium acetate and 3% acetic acid (p_H 4,8) was used.

Fresh apple leaf petioles were cut into pieces of approximately 1 mm. One gram of this cut material was extracted in 10 cc solution.

Dried apple leaves were as a whole ground in a Peppink mill. One gram of the powder was extracted in 100 cc solution.

Potassium, magnesium and phosphorus were determined in these extracts colorimetrically, with cobaltinitrite, titan yellow and ammonium molybdate, respectively.

Total analysis

For total chemical analysis the dried leaves were ground in the same way, in a Peppink mill.

P o t a s s i u m and m a g n e s i u m: One gram of powder was extracted during one hour in 50 cc 1 N HCl at 100°C. After cooling the extract was decolourized by shaking with charcoal. Neutralization was carried out by adding a fixed quantity of sodium hydroxide to 10 cc of the extract and diluting to 20 cc with Morgan's extracting solution. Thereafter the original extract was diluted 10 times. The results are expressed in mg per 100 g dry matter.

P h o s p h o r u s. One gram of powder was digested in a mixture of 1 part HNO_3 and 1 part H_2SO_4. After destruction the volume was made up to 50 cc. After neutralization with 50% NaOH and diluting with Morgans extracting solution, the solution had to be diluted in most cases 4 times. The results are expressed in mg P per 100 g dry matter.

All determinations of K, Mg and P were made in the same way as in the case of fresh leaves.

EXPERIMENTS

The first series of samples which suggested a study of this relationship, came from a river clay near Elst (Overbetuwe) a soil which is low in nitrogen, phosphate and potassium but high in magnesium. The trees growing on this soil showed symptoms of potassium deficiency.

As might be expected, the trees suffering from a potassium deficiency were associated with a low K/Mg ratio. This ratio, however, can vary within wide limits: in cases of severe magnesium deficiency it can be as high as 25–30 though normally it is of the order of 15–20.

Though there is little connection between manurial treatments and the corresponding nutrient status of the leaves, it is significant

that on all plots, the phosphate content is exceptionally high compared with the normal content, which at this time of the year is about 2 ppm.

TABLE I

Concentrations of K, Mg and P in Morgan's extracts of the petioles of apple leaves deficient in potassium. Experimental plot of the superphosphate company "Albatros" near Elst. Variety Cox on M IX; sampling date 20-9-'50

	Manurial treatment	ppm K	ppm Mg	ppm P	K/Mg
1	1K	90	70	12	1,2
2	1K + Mg	120	60	16	2,0
3	2K	180	60	8	3,0
4	2K + Mg	200	75	10	2,6
5	1K + Ca	80	80	14	1,0
6	1K + Mg + Ca	60	45	14	1,2
7	2K + Ca	80	60	10	1,3
8	2K + Mg + Ca	100	75	12	1,3
	Mean value	110	66	12	1,7

A survey of the phosphate contents of two varieties of apples on different root stocks during the period May to October gave the following results:

TABLE II

Seasonal variations in the P content of apple leaves as determined by extraction with Morgan's solution.

Date of sampling	ppm P, average of 30 samples
end of May	6,4
middle of June	5,5
,, ,, July	3,9
,, ,, August	2,5
,, ,, Sept.	1,8
,, ,, Oct.	2,2

More or less the same result was obtained from rapid colorimetric tissue tests made on samples from another experimental plot (Table III). In this case the potassium deficiency was not so severe though this was reflected more in the much lower magnesium contents of the leaves than in the potassium values which were similar to or even lower than those given in the first example. In accordance with these results the phosphate contents were much lower than in the foregoing experiment.

Again no significant effect of the manurial treatments could be observed. To obtain further evidence for a relationship between magnesium and phosphate status, analyses were made on material sampled from potassium deficient and magnesium deficient orchards, during the years 1947–1948.

TABLE III

Concentrations of K, Mg, and P in Morgan extracts of the petioles of apple leaves deficient in potassium
Experimental plot of Centraal bemestingsproefveld "De lange Ossekampen" Wageningen. Cox's Orange Pippin. Date of sampling 20-9'50

no. of plot	Manurial treatment	ppm K	ppm Mg	ppm P	K/Mg
A 8	+ K + N	60	18	3	3,3
10	+ K + P	70	21	3	3,3
13	+ Ca	60	21	6	2,8
14	+ Ca + N	70	18	4	3,8
B 2	+ N	70	18	4	3,8
8	+ K + N	100	15	4	6,3
14	+ Ca + N	60	21	6	2,8
20	+ N + Ca + K	80	15	3	5,3
	Mean value	71	18	4,1	3,9

The leaves were dried, ground in a Peppink mill and then analysed according to the Morgan method. Though the results obtained by this method cannot strictly be compared with those obtained using fresh material, comparisons can still be made between the two groups of dried samples. These again show a significant relationship between magnesium and phosphate contents.

Since it is well-known that the nitrogen nutrition also influences the phosphate status of the leaves, this difference in phosphate content may also be related to differences in nitrogen nutrition. The samples, however, were collected at random from potassium and magnesium deficient orchards which were on the whole well-looked after so that there was no reason to assume that the magnesium deficient samples were also deficient in nitrogen. The samples were all collected after the symptoms of potassium or magnesium deficiency had developed i.e. in August or September, so that at the time of sampling, the seasonal fall in phosphate content had taken place in all cases.

In order to check the applicability of the Morgan extracting

method, the same samples were analysed in another way. Approximate total contents of potassium and magnesium were obtained from N HCl (± 4%) extracts of the leaves.

TABLE IV

Concentrations of K, Mg and P in Morgan extracts (ppm) of dried potassium and magnesium deficient apple leaves.

Variety	K	Mg	P	K/Mg	Remarks
A) *Potassium deficient samples*					
1 Cox's Orange Pippin . . .	180	155	120	1,1	
2 ,, ,, ,, . . .	655	70	100	9,3	slight K-deficiency
3 ,, ,, ,, . . .	275	120	90	2,2	
4 ,, ,, ,, . . .	690	70	105	9,8	slight K-deficiency.
5 ,, ,, ,, . . .	685	90	100	7,6	,, ,,
6 ,, ,, ,, . . .	310	120	140	2,5	
7 Jonathan	240	110	140	2,1	
8 ,, ...	270	130	120	2,0	
9 ,, ...	350	140	180	2,5	
10 Laxton's Superb	285	115	100	2,5	
11 ,, ,, ...	400	165	120	2,4	
12 Beauty of Boskoop. . . .	310	120	120	2,5	
13 Lemoenappel	225	110	150	2,0	
Average *)	368	116	122	2,15	
B) *Magnesium deficient samples*					
1 Cox's Orange Pippin . . .	1000	175	80	5,7	slight deficiency
2 ,, ,, ,, . . .	2500	110	30	22,7	
3 ,, ,, ,, . . .	3000	150	55	20,0	
4 ,, ,, ,, . . .	1500	100	70	15,0	
5 ,, ,, ,, . . .	1100	175	40	6,2	,, ,,
6 Beauty of Boskoop. . . .	2000	140	35	14,2	
7 ,, ,, ,, ...	2700	30	40	90,0	
8 Laxton's Superb	1300	55	40	23,6	
9 Glorie van Holland. . . .	1400	45	60	31,1	
10 Manks Codlin	1000	150	40	6,6	,, ,,
11 James Grieve	1800	60	60	30,0	
12 Signe Tillisch	2000	60	40	33,3	
13 Sterappel en Tr. de Cr. . .	2300	40	60	58,5	
14 M type I	2200	60	130	36,6	
Average	1700	96	55	34,0	(nos 1, 5 and 10 excluded from average)

*) Average with the exception of nos 2, 4 and 5.

The total phosphorus content was determined after digestion of the material with a 1 : 1 mixture of concentrated sulphuric and

nitric acids. The following results were obtained:

TABLE V

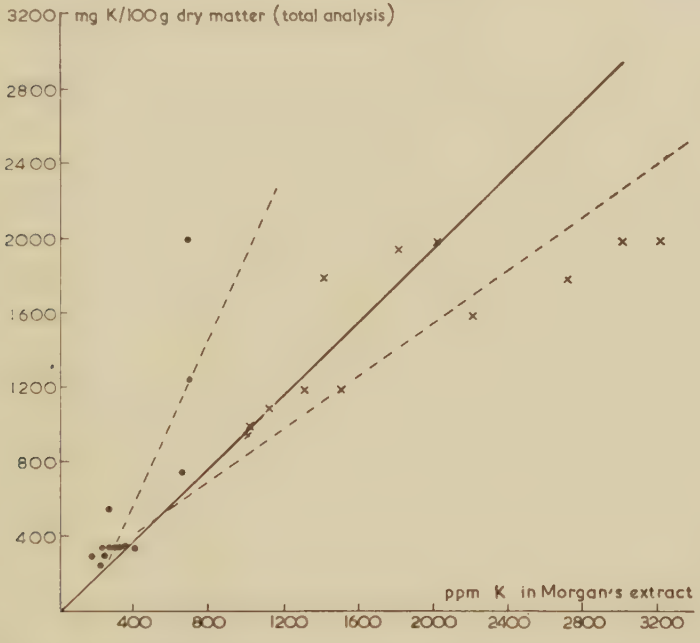
K, Mg and P contents of apple leaves determined in the HCl extracts (K and Mg) and after wet digestion *) (P). Figures expressed in mg/100 g dry matter

Variety	K	Mg	P	K/Mg	Remarks
A) *Potassium deficient samples*					
1 Cox's Orange Pippin . . .	300	600	250	0,5	
2 ,, ,, ,, . . .	750	200	250	3,25	only slight deficiency
3 ,, ,, ,, . . .	320	800	290	0,4	
4 ,, ,, ,, . . .	1250	160	170	7,8	,, ,, ,,
5 ,, ,, ,, . . .	2000	220	300	9,0	,, ,, ,,
6 ,, ,, ,, . . .	350	350		1,0	
7 Jonathan	350	370		0,9	
8 ,, ...	300	270		1,1	
9 ,, ...	550	300		1,8	
10 Laxton's Superb	320	320	255	1,0	
11 ,, ,, ...	325	350	275	0,9	
12 Beauty of Boskoop. . . .	350	320		1,9	
13 Lemoenappel	250	250	310	1,0	
Average	341	393		1,05	
(with the exception of nos. 2, 4 and 5)					
B) *Magnesium deficient samples*					
1 Cox's Orange Pippin . . .	1000	220	225	4,9	only slight deficiency
2 ,, ,, ,, . . .	1800	120	90	15,0	
3 ,, ,, ,, . . .	2000	120		16,6	
4 ,, ,, ,, . . .	1200	70	150	17,2	
5 ,, ,, ,, . . .	1100	200	180	5,5	
6 Beauty of Boskoop. . . .	—	—	—	—	
7 ,, ,, ,, . . .	2500	50	115	50,0	
8 Laxton's Superb	1200	50		24,0	
9 Glorie van Holland. . . .	1800	50	175	36,0	
10 Manks Codlin	950	150	115	6,3	
11 James Grieve	1950	50	135	39,0	
12 Signe Tillisch	2000	50	135	40,0	
13 Sterappel en Tr. de Croncels	1800	50	180	36,0	
14 M. type I	1600	50		32,0	
Average	1785	66	140	30,5	(nos 1, 5 and 10 excluded)

*) Method of Neumann-Fleischmann.

Comparing the results obtained for the potassium deficient samples, we see that the "total" determinations yield lower K/Mg ratios than do the Morgan's extracts. We can conclude therefore that errors associated with the latter method do not bias the results in

favour of the relationship expressed above. It is still not clear however, whether Morgan's solution extracts relatively less magnesium or whether the "total" method extracts relatively more magnesium from the tissues in proportion to the amount of potassium.



Graph I. The relation between the amounts of potash found with the extraction method and with total analysis.

The straight line is indicating where the points should ly in case both methods yielded the same result.

× : magnesium deficient samples

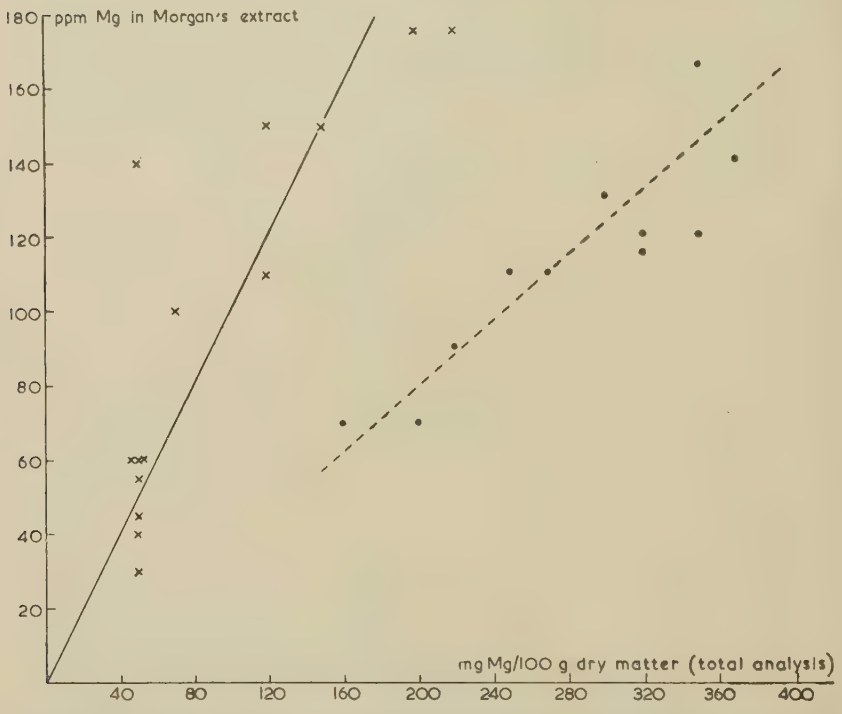
● : potash deficient samples

Though much less marked, the results obtained for the magnesium deficient samples show the same trend: in this case the greater deficiency in magnesium is reflected in a high K/Mg ratio.

Since ppm in table IV is the same as mg/100 g dry matter because of the dilution, the values can be compared on an absolute basis. For both series the figures can be considered to express mg per 100 g dry matter. In general we find much more magnesium and potassium using the total analysis method than with the extraction procedure but in certain cases of low magnesium content, total analyses give

lower magnesium values and also less potassium. As yet we have no explanation for this inconsistency.

Graphs I, II, and III illustrate the results obtained by the two



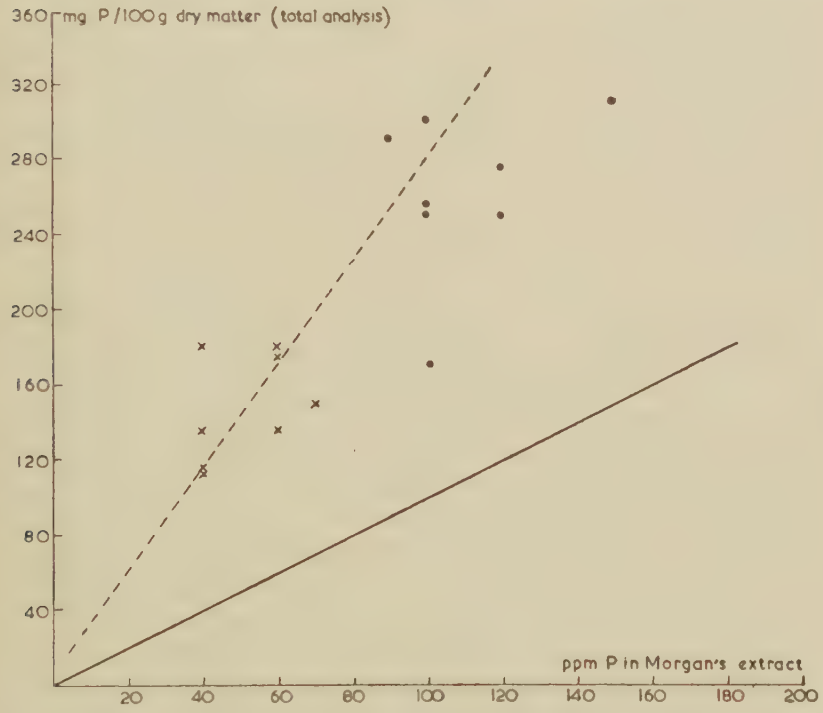
Graph II. The correlation between the amounts of magnesium determined in Morgan's extract and after total digestion. The drawn line gives the place of the points in case both methods give the same result.
× : cases of magnesium deficiency
● : cases of potash deficiency

methods of analysis. As far as phosphate is concerned, the values obtained by the total method are almost three times as high as those by the extraction method. For magnesium, the total method gives more than twice the content of the extraction method in the case of potassium deficient samples. The magnesium deficient samples show almost the same amount of magnesium with both methods.

From these data we may deduce that there is a positive correlation between the contents of magnesium and phosphate in apple leaves. This is of importance in connection with the phosphate

content of the soil and the amount of phosphate fertilizer applied to potassium and magnesium deficient soils.

Generally the potassium deficient samples were obtained from regions also low in phosphate. The magnesium deficient samples on the other hand were collected from a region where the phosphate content of the soil was relatively high. This would suggest that the



Graph III. The relation between the two methods of analysis for phosphorus ppm colorimetrically determined in Morgan's extract.

mg per 100 gr dry matter chemically determined after wet digestion.

The continuous line represents the place of the points in case of complete accordance between the two methods.

× : for magnesium deficiency

● : for potash deficiency

situation in the soil is just the reverse of that in the leaves. The application of very large amounts of phosphate to magnesium deficient soils is, therefore, of little value as far as the nutrition of the trees is concerned because the magnesium level does not permit the

uptake of more phosphate. It would appear much more appropriate to apply magnesium instead of phosphate.

DISCUSSION

Apart from the value of the above findings in connection with the nutrition of fruit trees, evidence has been obtained for the applicability of the Morgan method to nutrient investigations on these trees. Thus the parallelism between the results obtained by both Morgan's and the total analysis techniques enables one to treat the data obtained by the former method with more confidence.

In agreement with the work of B r o w n [3]) who found that apples with "bitter pit" had a high ash content and a low phosphate content, we have found in the leaves a high K content associated with a low phosphate content. This finding is of particular significance in view of the problems connected with bitter pit. It would appear that in cases of severe bitter pit, a high rate of magnesium fertilizer application would be of value provided that there is no acute deficiency in phosphorus due to a real shortage of this element.

As far as the application of phosphate fertilizers is concerned, it is of some importance to know that on certain soils high in potassium and low in magnesium, it may be more advantageous to fertilize with magnesium than with phosphate. It seems that the uptake of phosphate is governed so much by the balance between potassium and magnesium, that this balance may have an even greater influence than the actual amount of phosphate in the soil, at least above a level below which we get acute phosphate starvation. In the case of fruit trees at least, this level is seldom reached because of their low requirements for phosphate.

SUMMARY

1. Nutrient relations in apple leaves have been studied using Morgan's colorimetric method and a method for total analysis.

2. The results obtained by both methods are in agreement with each other.

3. Studies were made on the seasonal variation in the phosphate content of apple leaves.

4. In apple leaves of various varieties, a low potassium content is associated with high phosphorus and high magnesium contents.

5. This relationship has been demonstrated in comparisons between

samples from a potassium deficient river clay and a magnesium deficient marine loam soil.

6. The fact that high values for leaf phosphorus were obtained from soils with low phosphate contents and vice versa suggests that the phosphate content of the leaves is determined by factors other than the soil content.

7. From the parallelism between the phosphate and magnesium contents of leaves, it is concluded that magnesium plays an important role in the uptake of phosphate.

8. In order to increase the phosphate content of the leaves in magnesium deficient areas, it is probably more economical to apply magnesium rather than phosphate fertilizers.

9. Since, according to Brown, apples with bitter pit have a low phosphate content, the importance of the above relations in the case of the bitter pit problem is emphasized.

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BEITRÄGE ZUR PHYSIOLOGIE UND MORPHOLOGIE DER STREPTOMYCETEN

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Im Verlaufe unserer Untersuchungen über die Bedeutung der Bodenmikroorganismen, insbesondere Streptomyceten, für die Humusbildung war es notwendig, die zu untersuchenden Stämme in Bezug auf die Bildung der schwarzbraunen, den Huminsäuren sehr ähnlichen Produkte zu charakterisieren. Von einem Teil der genannten Mikroorganismenarten ist schon seit langem bekannt, daß sie auf Nährböden mit Pepton oder anderen eiweißhaltigen Stoffen ein dunkelbraunes bis schwarzes Pigment ausscheiden. Schon Beijerinck[1]) erkannte 1900 die Bedeutung dieser chromogenen Arten für die Humusbildung und hielt diesen Farbstoff auf Grund seiner chemischen Eigenschaften für ein Chinon. Münter[2]) und Krainsky[3]) bestätigten die Wirkung der organischen Stickstoffquellen für die Farbstoffbildung der Streptomyceten.

In neuerer Zeit wurden von verschiedenen Seiten Stoffwechseluntersuchungen angestellt, um Aufschluß über die Bildung dieser braunen Stoffe mit huminsäureähnlichem Charakter zu erhalten. Es sind hier vor allem die Arbeiten von v. Plotho[4]), Laatsch[5]) und Scheffer[6]) zu erwähnen.

Da wir auf Grund der bisher vorliegenden Ergebnisse der Überzeugung sind, daß nur ein größeres Versuchsmaterial einen Einblick in den Vorgang der Bildung von diesen den Huminsäuren ähnlichen Stoffen gewährt, begannen wir unsere Untersuchungen mit 42 verschiedenen Streptomycetenstämmen, die aus verschiedenen Vorkommen stammten und untersuchten zuerst ihre Fähigkeit, auf peptonhaltigen Nährböden dunkelbraune Stoffe auszuscheiden. Insgesamt waren von den 42 Stämmen 20 dazu in der Lage.

Auf Grund eingehender Untersuchungen kamen wir davon ab, mit Kulturlösungen zu arbeiten, die Pepton als Stickstoffquelle enthielten, da — wie wir nachweisen konnten — die schwarzbraunen, huminsäureweizähnlichen Produkte immer noch durch eiweißhaltige Stoffe verunreinigt waren. Weil Pepton chemisch keine einheitliche Substanz und es damit schwierig ist, genaue Untersuchungen des Stoffwechsels der Streptomyceten anzustellen, gingen wir zu einzelnen Aminosäuren über und untersuchten unsere Stämme auf folgenden Säuren:

1. Glykokoll
2. Sarkosin
3. Kreatin
4. Alanin
5. Serin
6. Threonin
7. Valin
8. Leucin
9. Isoleucin
10. Argininnitrat
11. Lysindihydrochlorid
12. Asparaginsäure
13. Glutaminsäure
14. Taurin
15. Cystein
16. Cystin
17. Methionin
18. Phenylalanin
19. Tyrosin
20. Kynurensäure
21. Tryptophan
22. Histidinmonohydrochlorid
23. Oxyprolin

Die einzelnen Aminosäuren kamen in Konzentrationen von 0,05; 0,1 und 0,2% als Stickstoff-Quelle zu einer Grundnährlösung mit

2,0%	Glycerin
0,2%	NaCl
0,1%	K_2HPO_4
0,005%	$MgSO_4$
0,002%	$CaCO_3$
0,001%	$FeSO_4$

Zu einem Teil der Kulturlösungen wurden die Aminosäuren auch in Mengen gegeben, die in Bezug auf ihren Stickstoffgehalt 0,2% Glykokoll äquivalent waren. Die Färbung der Kulturlösung war je nach Stamm und Aminosäuren sehr unterschiedlich. Das Wachstum wurde durchschnittlich 30 Tage beobachtet, da dann kaum noch eine Veränderung eintrat. Vollkommen farblos bzw. nur schwach gelblich blieben die Kulturlösungen aller Stämme bei Sarkosin, Kreatin, Alanin, Taurin, Cystein, Cystin, Kynurensäure und Histidin.

Von einer großen Anzahl von Stämmen wurde die Braunfärbung auf folgenden Aminosäure erreicht: Glykokoll, Serin, Threonin, Valin, Leucin, Isoleucin, Arginin, Lysin, Glutaminsäure, Methionin, Phenylalanin, Tyrosin, Tryptophan und Oxyprolin.

Die Braunfärbung setzte nach etwa 10–15 Tagen ein. Zuvor wurden verschieden gefärbte Zwischenstufen durchlaufen, die entweder vom grünlichen ins gelblich-rötliche oder vom gelblichen ausgehend ins braune übergingen.

Um zu einer gewissen Systematik unserer Untersuchungen zu gelangen, haben wir zunächst eine Zusammenstellung der die Nährlösung braunfärbenden Stämme gemacht. So lassen sich diejenigen Stämme, die sowohl Pepton als auch Aminosäure-Nährböden braun färben, hauptsächlich in drei Gruppen aufteilen.

Die erste Gruppe mit 3 Stämmen ruft vor allem auf Nährlösungen mit aliphatischen Aminomonocarbonsäuren Braunfärbung hervor.

TABELLE I

Gruppe I						
Stamm No.	1		2		3	
	Färbung	Wachstum	Färbung	Wachstum	Färbung	Wachstum
Pepton	braun		braun		braun	
Glykokoll	braun	+++	braun	++	braun	+
Serin	braun	+	braun	+	braun	++
Threonin	braun	++	braun	++	braun	+
Valin	braun	++	braun	+	braun	++
Leucin	braun	+	braun	+	braun	±
Isoleucin	braun	±	braun	±	braun	±
Lysin	braun	++	braun	++	braun	+++
Arginin	—	++	—	±	—	+++
Asparagins	—	±	—	++	—	++
Glutamins	—	++	—	++	—	+

Das Organismenwachstum steht in keinem Falle im Zusammenhang mit der Dunkelfärbung, wie aus der Tabelle I ersichtlich ist.

Oft findet trotz kräftigen Wachstums keine Dunkelfärbung statt und umgekehrt. Die folgenden Gruppen geben in Bezug auf Wachstum und Dunkelfärbung alle ungefähr das gleiche Bild. Es wurde daher bei den weiteren Tabellen von der Bezeichnung der Stärke des Wachstums abgesehen.

Die Gruppe II Stamm Nr. 4 bis 12 färbt diejenigen Nährlösungen braun, die einerseits Pepton und andererseits Arginin oder eine der beiden Aminodicarbonsäuren, Asparaginsäure und Glutaminsäure, enthalten.

Der Rest der Streptomyceten-Stämme lässt sich in vier weitere Gruppen aufteilen.

TABELLE II

	Stamm Nr.	Pepton	Glykokoll	Glutamins
Gruppe III	13—24	braun	braun	braun
Gruppe IV	15—20	braun	—	—
Gruppe V	21—26	—	braun	braun
Gruppe VI	27—42	—	—	braun

Die Gruppe III färbt sowohl Pepton als auch die Aminomonocarbonsäure Glykokoll und die Aminodicarbonsäure, Glutaminsäure braun.

Die Gruppe IV färbt nur Pepton braun. Während die Gruppe V und VI Pepton nicht verfärben, rufen sie einmal mit Glykokoll und Glutaminsäure oder zum anderen nur mit Glutaminsäure eine Braunfärbung hervor.

Um gewisse Zusammenhänge zwischen der Bildung der schwarzbraunen, mit Salzsäure fällbaren Produkte und dem physiologischen Verhalten der Streptomycetenstämme zu erfahren, gingen wir dazu über das Wachstum unserer Stämme auf solchen Nährböden zu beobachten, wie sie bei der systematischen Bestimmung allgemein üblich sind [7]) [8]) [9]).

In der Tabelle III ist das Wachstum der verschiedenen Stämme auf Haferflockenagar aufgezeichnet.

TABELLE III

Wachstum der Stämme auf Haferflockenagar			
	Stamm Nr.	Haferflockenagar	
		Sporen	Agarfarbe
Gruppe I	1—3	blau-grün	grau-schwarz
Gruppe II	4—11	weiß	farblos
	12	grau	braun
Gruppe III	13	grau	farblos
	14	blau	grau-schwarz
Gruppe IV	15—20	weiß-rosa	farblos
Gruppe V	21—26	grau-weiß	bräunlich
Gruppe VI	27—42	grau-weiß	bräunlich

In Gruppe I verhalten sich die Stämme ziemlich gleich. Die Sporenfarbe ist blaugrün und der Agar färbt sich grauschwarz. In Gruppe II, die eine weiße Sporenfarbe hat und den Agar nicht anfärbt, fällt der Stamm Nr. 12 heraus, der eine graue Sporenfarbe besitzt

und den Agar nur braun färbt. Ebenso zeigt der Stamm Nr. 14 ein unterschiedliches Verhalten gegenüber dem anderen Stamm. Die Sporen sind blau gefärbt und der Agar grauschwarz.

Gruppe V und VI, diejenigen Stämme, die Pepton nicht braun färben, verhalten sich auf Haferflockenagar ziemlich einheitlich.

Ihre Sporen sind grau bis weiß und der Agar wird nur schwach braun angefärbt.

In der nächsten Tabelle sind die Stämme 1, 2, 3 und 14, die sich ähnlich denjenigen der Gruppe I verhalten, noch weiteren physiologischen Prüfungen unterzogen worden.

TABELLE IV

Physiologische Prüfung der Stämme 1, 2, 3 und 14

Stamm	Kartoffel		KNO_3-Agar	
	Sporen	Verfärb.	Sporen	Verfärb.
No. 1	graugrün	braunviolett	bläulich	—
No. 2	grau	dunkelviolett	weißglänz.	—
No. 3	bräunlich	violett	weißgrau	—
No. 14	gräulich	violett	grau	bräunlich

	Stärke	Lakmus-Milch		Gelatine	
	Sporen	Verfärb.	Flock.	Verflüss.	Verfärb.
No. 1	graugrün	dunkelgrau	+	++	braun
No. 2	graugrün	dunkelgrau	—	++	braun
No. 3	graugrün	graubraun	+	++	braun
No. 14	grau	bräunlich	++	+++	braun

Auch bei der Kultur auf Kartoffelscheiben lassen sich sowohl in der Sporenfarbe wie auch bei der Verfärbung der Kartoffeln verschiedene Unterschiede erkennen.

Sehr unterschiedlich verhalten sich diese Stämme sowohl in Sporenfarbe wie auch in der Verfärbung des Kulturmediums auf Kaliumnitrat-Agar. Auch auf Stärke zur Prüfung der Amylasewirkung bestehen gewisse Unterschiede.

Sie zeigen ferner ein unterschiedliches Verhalten gegenüber Lakmusmilch; diese wird sehr verschieden gefärbt und die Gerinnung ist verschieden stark bzw. unterbleibt bei verschiedenen Stämmen.

Bei der Prüfung mit dem Gelatinestich besteht nur ein gewisser Unterschied bei der Verflüssigung der Gelatine; die Verfärbungen sind nahezu gleich.

Da es bei unseren Untersuchungen letzten Endes auf die Zusammensetzung der ausgeschiedenen huminsäureähnlichen Stoffe an-

kam, erhofften wir mit mehr physikalisch-chemischen Methoden eine Möglichkeit zu finden, um sowohl die Sporenfarbe wie auch das Kulturmedium, in das die gesuchten Stoffe ausgeschieden wurden, zu charakterisieren. Wir beobachteten daher die Fluoreszenz sowohl der Sporen wie auch des Nährbodens bei der Kultur auf Pepton- bzw. Haferflockenagar. Es war in diesem Falle eine Ähnlichkeit einigermaßen zu erkennen.

TABELLE V

Stamm	Fluoreszenz			
	Pepton		Haferflocken	
	Sporen	Agar	Sporen	Agar
No. 1	schwachrot	+ grün	schwachrot	—
No. 2	schwachrot	++ grün	rot	—
No. 3	—	+ grün	—	—
No. 14	schwachrot	+ grün	rot	+ schwachblau

Auf Peptonagar war nur eine schwachrote Fluoreszenz der Sporen zu erkennen, während hingegen alle Nährböden, in die die schwarzbraunen Produkte eingedrungen waren, eine mehr oder minder starke, grüne Fluoreszenz zeigten.

Auf Haferflockenagar war die Fluoreszenz der Sporen fast bei allen Mikroorganismen rot. Die Nährböden dagegen zeigten keine Fluoreszenz.

Selbst diese Untersuchungen ergaben keinen systematischen Zusammenhang zwischen dem Stoffwechsel und der Ausscheidung schwarzbrauner Produkte.

Wir haben nämlich sowohl beim Gelatinestich wie auch bei der Fluoreszenz auf Pepton — da diese Messungen bei allen Stämmen am gleichartigsten erschienen — alle anderen Gruppen darauf untersucht, ob nicht diese Eigenschaften auch von denjenigen Stämmen gegeben werden, die wir vorher auf Grund des Wachstums auf Pepton oder verschiedenen Aminosäuren eingeteilt hatten. Wir konnten dabei folgende Feststellung treffen:

TABELLE VI

Gruppe	Gesamtzahl	Gelatine-Verflüss.	Fluoreszens Pepton
II	7	7	4
IV	6	5	3
V	6	1	3
VI	15	4	6

In der Gruppe II verflüssigen von sieben Stämmen alle die Gelatine und vier zeigen die gleiche Fluoreszenz auf Peptonagar wie in der obigen Aufstellung angegeben ist. Von Gruppe IV verflüssigen die Gelatine fünf und drei zeigen die gleiche Fluoreszenz von sechs Stämmen. Von Gruppe V, die Pepton nicht braun färbt, jedoch Glykokoll und Glutaminsäure, verflüssigt ein Stamm die Gelatine und drei zeigten die gleiche Fluoreszenz von sechs Stämmen. Von Gruppe VI, die ebenfalls Pepton nicht braun färbt sondern nur Glutaminsäure, zeigen von fünfzehn Stämmen vier eine Gelatineverflüssigung und sechs die gleiche Fluoreszenz auf Peptonnährböden.

Nachdem die physiologischen Untersuchungen kein klares Bild über den Zusammenhang zwischen den physiologischen Eigenschaften und der Bildung der schwarzbraunen Produkte ergaben, gingen wir dazu über, nach morphologischen Merkmalen zu suchen. Wir haben zu diesem Zweck die Sporen sämtlicher Stämme mit dem Elektronenmikroskop geprüft. Es stellte sich bei dieser Untersuchung heraus, daß im Anklang an die physiologische Prüfung auf Haferflockenagar auch hier sich etwa die gleichen Eigenschaften erkennen lassen.

Bei einer 1600fachen Vergrößerung mit dem Elektronenmikroskop zeigten die Streptomycetensporen im Prinzip kein anderes Bild als mit dem Lichtmikroskop. Die äußeren Umrisse sind infolge der elektronenoptischen Technik jedoch besser zu erkennen (Abb. 1).

Geht man jedoch mit der Vergrößerung herauf, so kann man feststellen, daß diejenigen Stämme, die auf Haferflockenagar eine mehr oder minder blaue Farbe zeigen und den Agar grauschwarz anfärben, nahezu die gleiche Sporenform haben. Es waren dieses die Stämme 1 bis 3 aus Gruppe I, sowie der Stamm 14 aus Gruppe II. Diese Sporen zeichnen sich dadurch aus, daß sie an ihrer Oberfläche stachlige Fortsätze haben, die je nach Stamm kleine Unterschiede zeigen (Abb. 2 und 3).

Daß diese stachelähnlichen Fortsätze keine Zufallsprodukte durch die Bedingungen im Elektronenmikroskop sind, geht aus der Bedampfung des Objektes mit Platin hervor. Es lassen sich die Stacheln im Elektronenschatten sehr gut erkennen.

Bei der Beobachtung im Elektronenmikroskop kann man auch feststellen, daß die Sporen die Stacheln verlieren können (siehe Abb. 3).

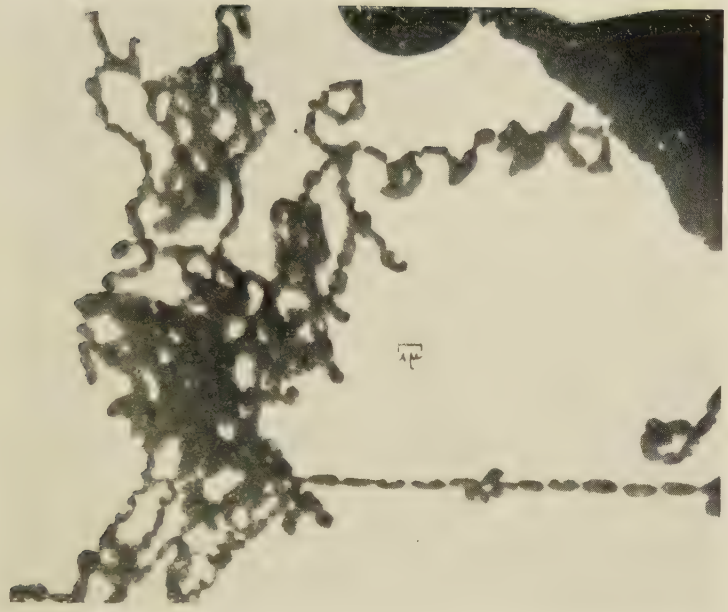

Abb. 1. Sporen Stamm No. 1
Org. Vergr. 1 600

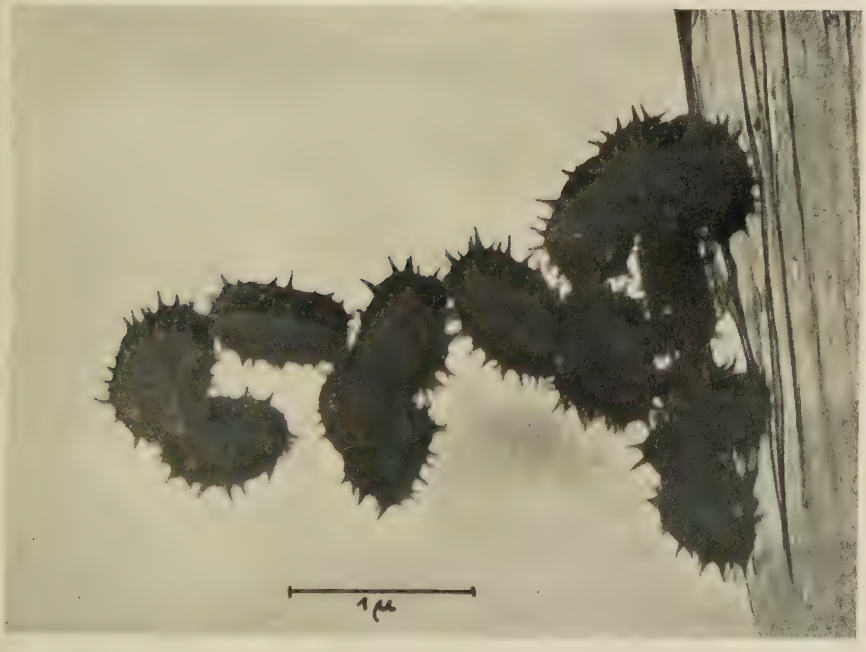

Abb. 2. Sporen Stamm No. 1
Org Vergr. 6 000

Abb. 3. Sporen Stamm No. 3
Org. Vergr. 6 000

Abb. 4. Hyphen Stamm No. 3
Org. Vergr. 6 000

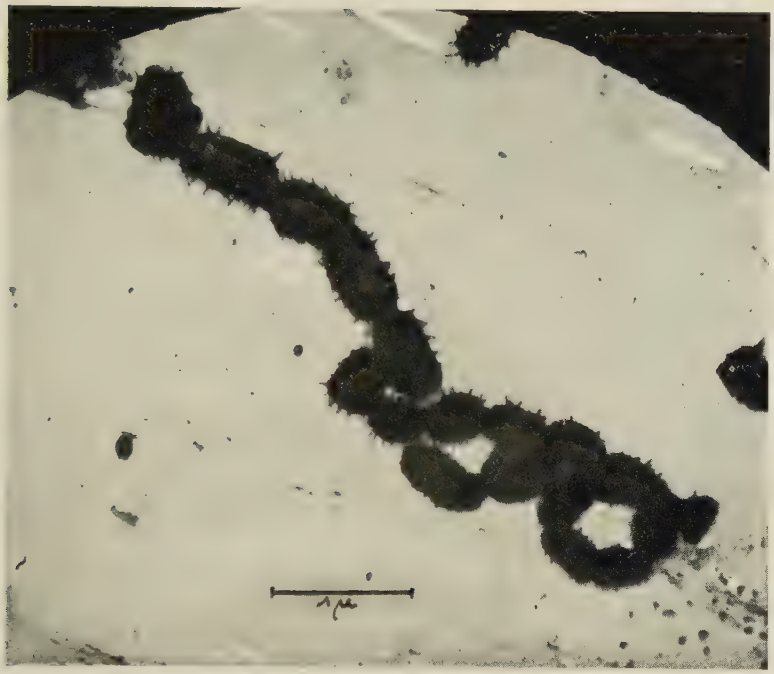

Abb. 5. Sporenkette Stamm No. 3
Org. Vergr. 6 000

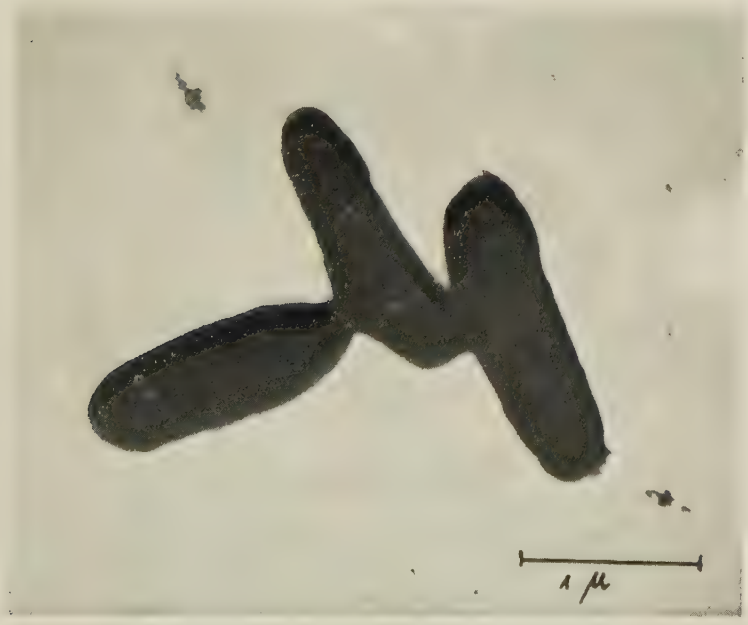

Abb. 6. Sporen Stamm No. 6
Org. Vergr. 6 000

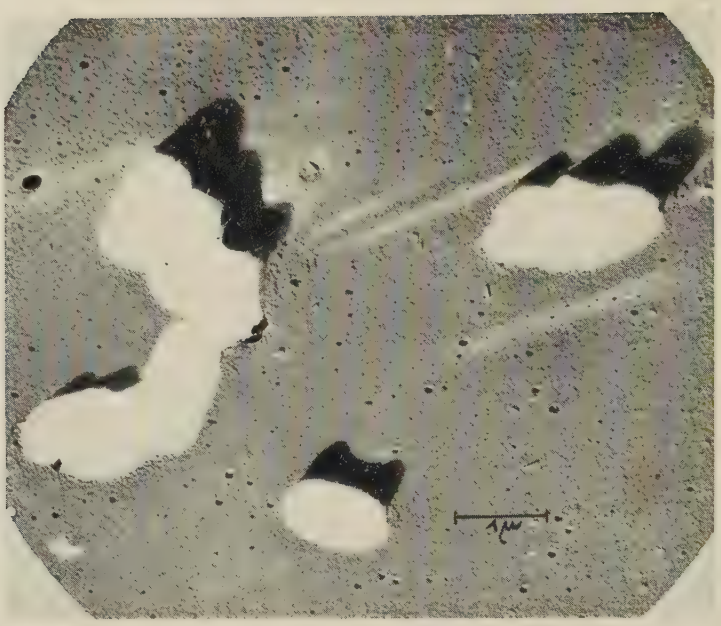

Abb. 7. Sporen Stamm No. 10 mit Platin bedampft
Org. Vergr. 6 000

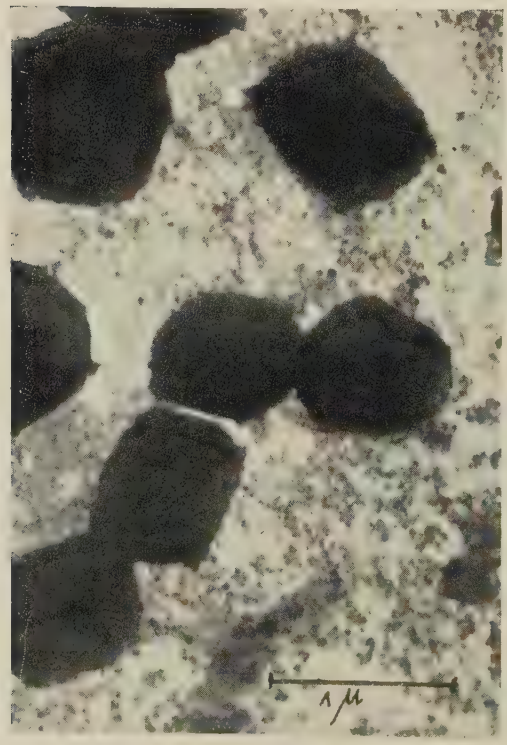

Abb. 8. Sporen Stamm No. 16
Org. Vergr. 6 000

Aus diesen Aufnahmen geht hervor, daß die Stämme sich wohl sehr ähnlich sind, aber es sich keineswegs um ein und denselben handeln kann, da diese außer der etwas verschiedenen Form der Sporen auch verschiedene physiologische Eigenschaften besitzen.

Wir haben weiterhin damit begonnen, uns mit der Sporenbildung der Streptomyceten zu befassen. Die bis jetzt gefundenen Ergebnisse sind noch nicht erschöpfend und sollen hier nur angedeutet werden. Bei verschiedenen Aufnahmen lassen sich hyphenähnliche Gebilde erkennen, die mit Stacheln besetzt sind. Diese schnüren sich ein und aus den eingeschnürten Anteilen entstehen dann in Form von Ketten die Sporen, die später weiter in Einzelsporen zerfallen (Abb. 4 und 5).

Bei der Durchsicht der Sporen der Stämme der Gruppe II kann man eine längliche, ovale Form als Charakteristikum angeben.

Einen typischen Vertreter zeigt die Abbildung 6, es sind die Sporen von Stamm Nr. 6.

Die Form der Sporen des Stammes Nr. 10 in Gruppe II ist nicht so sehr in die Länge gezogen.

Um die Sporen besser zu erkennen, haben wir sie auch in diesem Falle mit Platin bedampft.

Aus den Schattenkontrasten ist die ovale Form sehr gut zu erkennen. Die untere Spore scheint auf Grund des Schattens eine Einbeulung zu haben (Abb. 7).

Die Stämme der Gruppe IV scheinen sich wiederum durch eine andere Sporenform auszuzeichnen. Diese haben mehr tonnenförmige Gestalt (Abb. 8, Stamm No. 16).

Wir haben auch in diesem Falle versucht, auf Grund der Schattenprojektion die Gestalt dieser Sporen zu rekonstruieren.

Es gelingt auf diese Weise die Größenverhältnisse der Sporen wenigstens einigermaßen zu bestimmen (Abb. 9).

Auf einer anderen Aufnahme gelang es uns direkt, die tonnenförmige Gestalt der Sporen des Stammes Nr. 15 nachzuweisen.

Auf der Abb. 10 ist deutlich zu erkennen, daß die Sporen an ihren Enden abgeflacht sind und zum Teil sogar eine Verdickung zu haben scheinen.

Die Sporen der Gruppe V und VI, die Pepton nicht anfärben, hatten meist eine kugelförmige Gestalt. Auch hiervon sind nur einige charakteristische Aufnahmen wiedergegeben.

In diesem Falle wurde ebenfalls die Bestimmung der Gestalt etwas

ausführlicher durchgeführt. Die Abbildung 11 ist eine Rekonstruktion der Gestalt der Sporen des Stammes Nr. 22 auf Grund des Schattenbildes.

Auf der folgenden Aufnahme ist die Entstehung der Sporen von Stamm Nr. 25 zu sehen, wie sie gerade noch in einer Kette zusammenhängen (Abb. 12).

Die Sporen der Gruppe VI unterscheiden sich kaum von denen der Gruppe V und sind ebenfalls nahezu kugelförmig.

Die Aufnahmen der Sporen wurden von Kulturen gemacht, die nahezu das gleiche Alter hatten. Die Untersuchungen der Sporen von Kulturen in einem Zeitraum zwischen 1 und 12 Monaten ergab immer deren gleiche Gestalt. Die Gestalt der Sporen ändert sich daher nicht mit der Kulturdauer.

Wir sind uns darüber im klaren, daß die morphologischen Bestimmungen der Sporen durch die elektronenmikroskopischen Aufnahmen noch nicht den Anspruch erheben dürfen, eine endgültige Klärung in der Systematik der Streptomyceten zu geben. Diese Aufnahmen sind jedoch der erste Schritt zu weiteren Untersuchungen. Vor allem deuten die Befunde bei denjenigen Stämmen, die auf Haferflockenagar blau sporulieren und eine Braunfärbung des Agars hervorrufen, einen erfolgversprechenden Weg für die elektronenoptischen Untersuchungen an.

Vielleicht besteht auch einmal die Möglichkeit, diejenigen Stämme, die Antibiotica bilden, in Kombination mit physiologischen Merkmalen und auf Grund ihrer Sporenform elektronenmikroskopisch näher zu beschreiben.

ZUSAMMENFASSUNG

Auf Grund des Wachstums von Streptomycetenstämmen auf einer größeren Anzahl von Aminosäuren, der dabei auftretenden Braunfärbung und des Verhaltens auf verschiedenen Nährböden, sowie auf Grund der morphologischen Untersuchung der Sporen durch das Elektronenmikroskop, sind Anhaltspunkte für eine Einteilung der Streptomyceten in verschiedene Gruppen mit gleichen Eigenschaften erhalten worden.

Eingegangen 16 Oktober 1951.

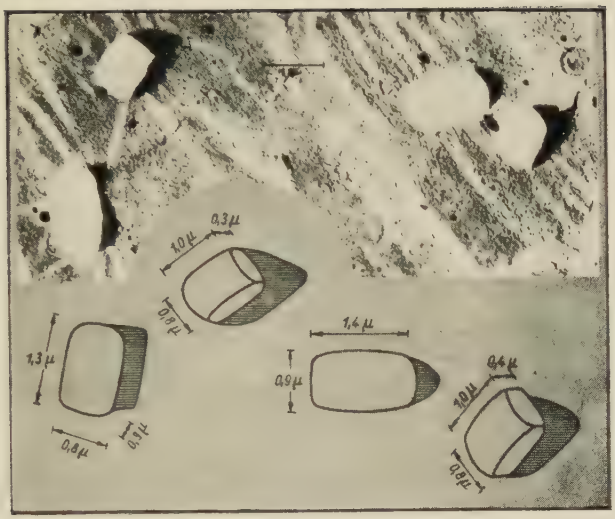

Abb. 9. Schattenkonstruction Sporen Stamm No. 16

Abb. 10. Sporen Stamm No. 15
Org. Vergr. 6 000

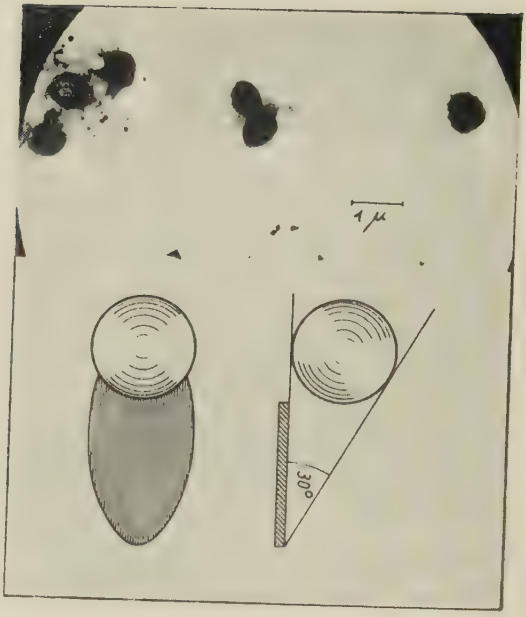

Abb. 11. Schattenkonstruktion Sporen
Stamm No. 22

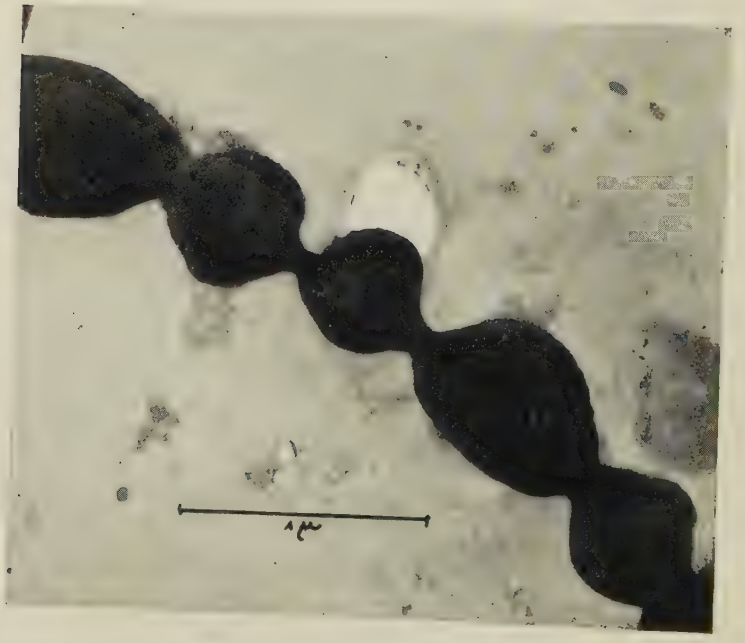

Abb. 12. Sporen Stamm No. 25
Org. Vergr. 16 000

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CENTROSPORA ROOT ROT OF *PRIMULA MALACOIDES*, A SERIOUS SOIL-BORNE DISEASE IN DENMARK

by PAUL NEERGAARD

Copenhagen

During the last ten years *Centrospora acerina* (Hartig) Newhall *) has increasingly attracted the attention of plant pathologists. This fungus is now known to occur as a parasite on a great number of hosts in Europe and America. Neergaard and Newhall[5]) give a list of 23 species, hitherto reported as naturally infected hosts in the field, and a further 30 species which have been infected experimentally by various investigators.

In Denmark the fungus has been reported from parsley (Neergaard[1]), *Viola tricolor* (Neergaard[2])), *Primula malacoides* (Neergaard[3])) and *Primula veris* (Neergaard[4])).

The occurrence of *Centrospora acerina* on *Primula malacoides* has been associated with a root rot causing a serious reduction in the output of this important commercial crop. The aim of the present investigations has been to establish the cause of the root rot, suspected to be due to *Centrospora*, and to find suitable control measures against the disease.

Most of the experiments were carried out in the nursery of Ove K. Nielsen of Glostrup, Copenhagen, and I wish to render my thanks to Mr. Nielsen for his cooperation whilst the experiments were being carried out. I also wish to thank Mrs Jytte Hjo Taplov, B.S.Hort., for valuable technical help.

1. ECONOMIC SIGNIFICANCE

The root rot of *Primula malacoides* is known to have occurred in the nursery of Ove K. Nielsen every year since 1932 on

*) Synonyms: *Cercospora acerina* Hartig, *Centrospora ohlsenii* Neerg., *Ansatospora macrospora* (Osterw.) Newhall. For other synonyms see Neergaard and Newhall 1951.

several thousands of pot grown plants. In the winter of 1944–45 more than 50 per cent of the plants were killed which, according to the grower, involved a loss of about 25.000 kr. In the seasons 1946–47, 1947–48 and 1948–49 the disease was responsible for losses varying from 12.000 to 20.000 kr. — in 1948–49 25 per cent of the plants were killed.

This instance is undoubtedly not an isolated case. In April 1951 I was informed that heavy attacks of a root rot of *Primula malacoides* had occurred over a period of several years in at least three nurseries in Copenhagen, and descriptions of the symptoms agreed well with those of *Centrospora* root rot. In one of these nurseries the growing of *Primula malacoides* was abandoned on account of the heavy losses due to the disease. Unfortunately I became aware of these attacks too late for an examination of diseased plants from any of these places to be possible.

2. ETIOLOGY

Centrospora acerina has been thoroughly described and illustrated by Westerdijk and v. Luijk [9], by Osterwalder [7], by Neergaard [1] and by Newhall [6]. Tompkins and Hansen [8] give a good illustration of the conidia.

The morphological and cultural characteristics of isolates from *Primula malacoides* have been compared with those of isolates from various other host species. Isolates from the following other host species have been studied: *Primula veris*, *Petroselinum crispum var. radicosum*, *Apium graveolens* and *Daucus carota*.

The isolates from these hosts agreed perfectly with regard to cultural characters as well as morphology of the hyphae, conidiophores and conidia. The most important morphological characters can be summarized as follows (for details reference should be made to the authors mentioned above):

The resting mycelium is composed of brown, septate hyphae, 6–12 μ in width, the cells of which are about 15 μ long. In older hyphae the cells are frequently more or less spherically thickened (chlamydospores) and such hyphae easily break at the septa. The conidia are hyaline, protracted obclavate, gradually narrowing and becoming threadlike towards the apex, 6–12-septate and 105–210 $\times$ 6–11 μ in size. Most of the conidia have a sword- or spurlike (hence, *kentron*,

spur, in *Centrospora*) appendage, 0–2-septate, 30–150 × 2–3 μ, hanging down from the basal cell (fig. 1).

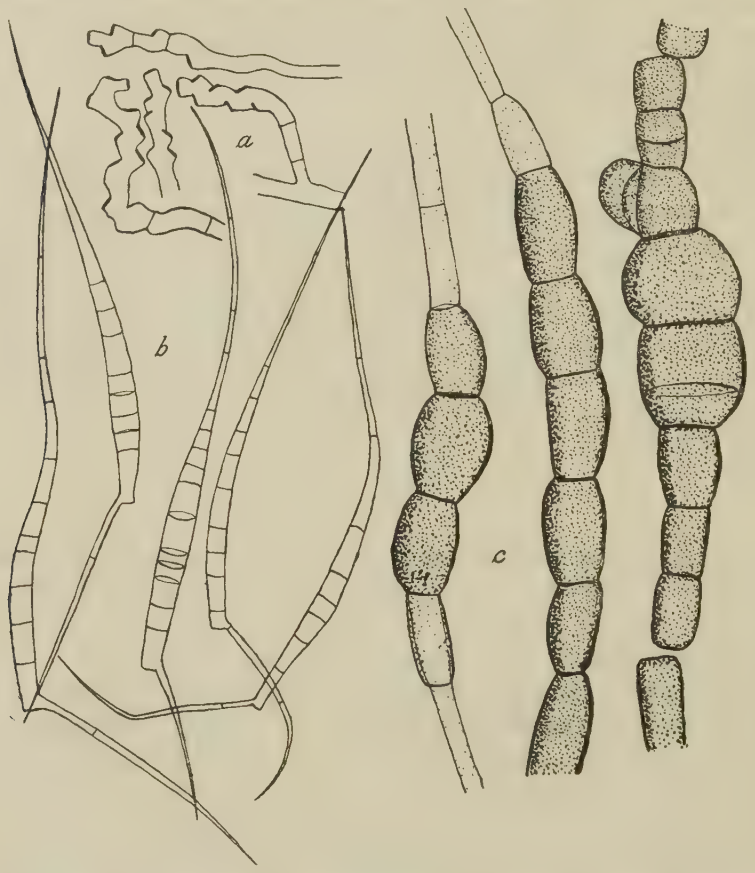

Fig. 1. *Centrospora acerina*. *a*. Conidiophores, *b*. conidia, *c*. resting mycelium (chlamydospores). Magnif. ×600 (After Neergaard 1942).

3. PATHOGENECITY

A. *Infection experiments*

a. Isolation. The characteristic resting mycelium of *Centrospora* could easily be found in the roots of diseased plants. This mycelium was especially abundant in the thick main root which was always found to be almost totally destroyed in plants which had been attacked and even in plants where most of the smaller roots appeared healthy.

The fungus was readily isolated from infected roots of *Primula malacoides* by removing small pieces of resting mycelium with an inoculation needle. Isolate I (isolated March 11, 1948) was obtained from a single piece of resting mycelium. Isolate II (isolated February 14, 1949) and the reisolate (isolated February 6, 1950) are single-spore isolates. Pieces of resting mycelium and small fragments of root tissue were taken from infected plants and placed together in

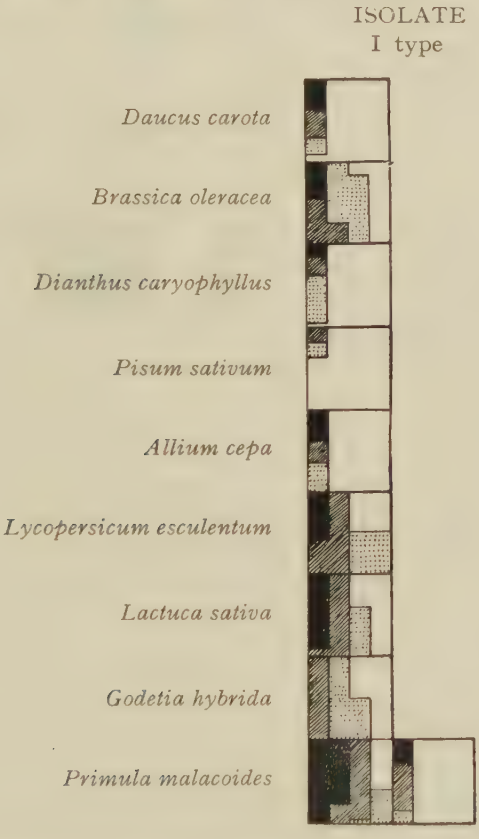

Fig. 2. Extent of infection in seedlings of nine hosts, grown under sterile conditions, 12 days after inoculation with *Centrospora acerina*.

drops of water on sterile slides for 2 days. Conidia developed profusely on the surface of the drops, and from this sporulation single spore isolates of the fungus were obtained.

b. Inoculation. In the infection experiments pot grown plants were inoculated by placing pieces of about 10 day old malt

extract agar cultures of the fungus in the middle of the root ball with the aid of a sterile scalpel. Sterile malt extract agar was placed in the root ball of a number of control plants in precisely the same way.

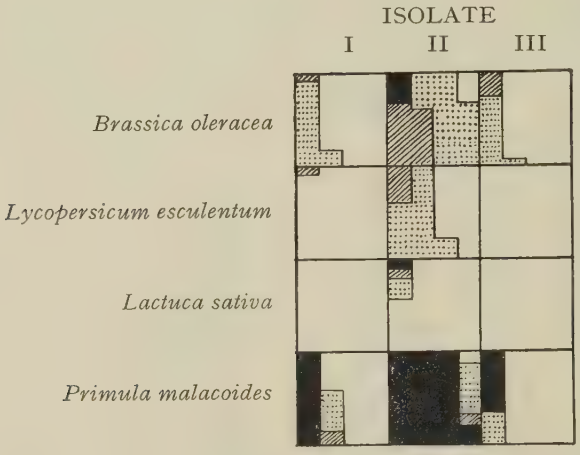

Fig. 3. Extent of infection in seedlings of four hosts, grown under sterile conditions, 12 days after inoculation with three isolates of *Centrospora acerina*.

c. Infection. Infection experiments with the above mentioned isolates I and II from *Primula malacoides*, isolate III from *Primula veris* (isolated December 20, 1948) and an isolate of *Petroselinum crispum* var. *radicosum* (type culture of the genus, described by Neergaard 1942) were carried out using sterile seedlings in test tubes.

Figures 2 and 3 summarize the results of these experiments on the basis of the infection percentages obtained — details of the results are recorded in another publication (Neergaard and Newhall[5]). In the diagrams the size of the black areas indicates the proportion of plants killed, lined areas those severely attacked, and dotted areas those slightly attacked. All of the nine species tested were more or less susceptible, and *Primula malacoides* was inoculated with all the four isolates with positive results.

The first infection experiment with pot plants included 40 plants inoculated with the fungus and 20 plants treated, as described above, with sterile agar. The plants were inoculated on December 21, 1948. They originated from seed sown in June 1948, the seedlings were pricked out in July and potted in August. 10

inoculated and 5 agar-treated plants were placed in each of 4 cold frames, together with a number of untreated plants.

On February 4, 1949, 14 of the 40 *Centrospora*-inoculated plants were killed, *i.e.* 35 per cent, whereas 3 of the 20 agar-treated plants were killed, *i.e.* 15 per cent. Of the untreated plants, comprising about 5000 plants, about 20 per cent were spontaneously attacked. Although there is some evidence of a positive result from the inoculations, no definite conclusion could be drawn from this experiment.

A second infection experiment was performed on September 13, 1949. All the plants used in this experiment were potted in steam-sterilized soil, but some of the plants for this experiment came from an experiment concerned with chemical treatment of the soil. The plants in question had been pricked out in unsterilized soil, and the soil used later for potting the seedlings had been treated with a fungicide, Agranin (see the chapter dealing with the experiments on control measures.) These plants were included in the infection experiment in order to see whether the fungicide would impede the development of the fungus, inoculated after the chemical treatment of the potting soil.

The results of this experiment are recorded in Table I.

TABLE I

Experiment on the infection of pot grown plants of *Primula malacoides* with *Centrospora acerina* performed in September 1949

Treatment of the plants	Inoculation Sept. 13, 1949	Number of plants	Records Jan. 24, 1950	Records Febr. 28, 1950
Plants pricked out in unsterilized soil, potted in sterilized soil treated with Agranin	Inoculated with Centrospora	7	5 attacked 2 healthy	6 acttacked 1 healthy
	Treated with sterile agar	4	4 healthy	1 attacked 3 healthy
Plants pricked out and potted in sterilized soil	Inoculated with Centrospora	11	8 attacked 3 healthy	10 attacked 1 healthy
	Treated with sterile agar	6	6 healthy	6 healthy

From this table it appears that at the time of the record, in January 1950, 72 per cent of the inoculated plants were attacked, but none of the plants treated with sterile agar were attacked. At the end of February, 89 per cent of the inoculated plants were infected, whereas only 1 of 10 plants treated with agar was infected and even this was

one of the plants pricked out in unsterilized soil. Fig. 4 illustrates the results obtained in this experiment. The plants treated with sterile agar show a slight reduction in vigour which is the result of disturbance of the root ball associated with the treatment.

In a third infection experiment plants which had been grown entirely in steam-sterilized soil were inoculated on February 28th 1950 with a reisolate of the fungus. The results are recorded in Table II.

TABLE II

Experiment performed in February 1950 on the infection of *Primula malacoides* in pots, with *Centrospora acerina*

Inoculation Febr. 28, 1950	Number of plants	Records March 14, 1950	Records April 3, 1950	Percentage infected
Inoculated with *Centrospora*	15	6 attacked 9 healthy	13 attacked 2 healthy	87
Treated with sterile agar	15	15 healthy	15 healthy	0
Untreated	20	20 healthy	20 healthy	0

B. *Symptomatology*

In experiments using sterile seedlings grown in test tubes the fungus has proved able to produce the usual symptoms of damping-off, a brown discolouration of the root and hypocotyl and a shrivelling of the tissues — within a few days the seedling may be killed. The disease has so far not been observed on seedlings growing under natural conditions.

In the case of full-grown plants the fungus causes a rot of the roots and base of the stem whereas primary rot symptoms on the leaves and petioles have not been observed. In the nursery where the present investigations were performed during the last four seasons, it has been observed that practically all the plants remain healthy until the power of the sun increases in the spring. When the plants approach the flowering stage they have probably been slightly infected for a long time, maybe for several months. The increased transpiration from the leaves, brought about by the spring sun, requires increased activity of the roots. In some cases diseased plants may succumb in the course of only a few days, in other cases weakening of the plant can be detected from the appearance of the top some weeks before the plant collapses. In this last phase of the disease

Fig. 4. Experiment on the infection of *Primula malacoides* with *Centrospora acerina*. Each treatment represented by 2 plants above, 4 plants below. From left to right: Inoculated with the fungus, inoculated with sterile agar, untreated. The plants treated with sterile agar have a slightly lighter leaf colour than the untreated on account of the disturbance caused in the root ball by the inoculation.

Fig. 5. Roots of *Primula malacoides* spontaneously attacked by *Centrospora acerina*. Left: Several of the larger roots in the centre of the ball are attacked, most of the other roots fresh. Right: The main root severely rotted, while practically all of the other roots appear healthy.

before collapse the first indication of the presence of the parasite is a remarkable change of leaf colour. At first the leaves begin to lose their normal fresh green colour and become slightly chlorotic with a characteristic muddy greenish tint. The leaves and petioles then gradually become softer and wilting commences, finally the whole plant succumbs.

These symptoms of Centrospora root rot should not be confused with those of another disease rather frequently encountered on *Primula malacoides*, namely Botrytis rot (*Botrytis cinerea* Pers.). In this disease a soft rot develops in the petioles and leaves and often in the heart. As a rule the plant parts attacked are soon covered with a characteristic grayish coat of the sporulating fungus. The leaves do not normally lose their fresh colour before the fungus has attacked the leaf tissues.

As soon as the attack of *Centrospora* can be discerned by the symptoms occurring the top of the plant, the presence of the fungus in the root tissues can be easily demonstrated. As mentioned above, the main root in such plants is always more or less rotten and resting mycelium of the fungus is present in abundance in the decomposing tissues. Whereas a number of the larger roots may be completely rotten, very often most of the smaller roots remain unaffected. The fungus grows through one or more of the main roots into the base of the stem thus greatly interfering with water movement (see fig. 5).

C. *Differences in susceptibility*

There are considerable differences between varieties of *Primula malacoides* with regard to susceptibility to the disease.

A variety having light rose flowers was heavily attacked and was used for the experiments dealt with here, whereas a variety with rose flowers of a darker and more brilliant hue was attacked less. The names of the varieties cannot be reported for commercial reasons.

4. CYCLE OF THE DISEASE

Attempts to isolate the fungus from soil in which *Primula malacoides* has grown, have failed whether the plants were healthy or attacked by *Centrospora acerina*. On the other hand the fungus has been recovered from muck soil of two counties in New York State where celery had been attacked (N e e r g a a r d and N e w h a l l 1951). The results reported below from the experiments on steam

treatment of the soil to be used for *Primula* growing makes it obvious that the soil is the important source of infection.

In Denmark the seed of *Primula malacoides* is usually sown in June, the seedlings are pricked out in July and finally potted in August. The plants are sold during the first 3 months of the following year. The disease has not been observed in the seedlings or young potted plants. In these stages of the growth of the host the inoculum is present in the soil or, probably, as a weak infection in the roots. During the period February–March, just before or at the commencement of flowering, a root rot develops and sooner or later the host is killed before flowering has finished.

Probably the fungus is not seed-borne in the case of *Primula malacoides* or, if so, it occurs extremely seldom on the seed. It has never been found on the seed by examination of more than 50 seed lots during the last 5 years and seed disinfection did not prevent the disease in the nursery where these investigations have been carried out.

5. CONTROL

A. *Laboratory experiment with chemicals*

An *in vitro* experiment was carrried out in which four chemicals were tested as to their effect on *Centrospora acerina*, *Rhizoctonia solani* Kühn and *Alternaria brassicicola* (Schwein.) Wiltsh. The chemicals used were: Germisan (5.8 per cent phenylmercuripyrocatechol, *i.e.* 3 per cent mercury), Agranin *) (3.7 per cent methoxyethylmercurichlorid, *i.e.* 2.5 per cent mercury), blue vitriol and hydroxyquinoline sulphate.

The fungi were grown in test tubes (about 20 mm in diameter) on Knops' nutrient solution having a reaction of pH 5.2. For each chemical two test tubes were used for each of five concentrations. The solutions were autoclaved.

The results, based on the effect on the amount of growth of the fungi, as recorded 6 weeks and 9 weeks after the transference to the test solution may be summarized as follows.

Hydroxyquinoline sulphate had about the same effect on the three organisms as the active factor, Hg, in Germisan and Agranin.

Growth of the mycelium of *Rhizoctonia* was inhibited at concentrations of $^1/_4$ p.p.m. of Hg, $^1/_2$ p.p.m. of hydroxyquinoline sulphate,

*) Supplied by the firm Ferrosan, Copenhagen. Not on the market.

while the highest concentrations of blue vitriol, 8 p.p.m., did not inhibit growth.

Alternaria showed little tendency to grow submerged in the liquid. The vigorous growth of mycelium on the surface of the liquid from the floating inoculum may have hindered the submerged growth. This difficulty made the results difficult to assess. Growth was inhibited at a concentration of about 4 p.p.m. of hydroxyquinoline sulphate, while the highest concentrations of Hg, $^1/_2$ p.p.m., and of blue vitriol, 8 p.p.m., did not fully inhibit growth.

Finally, the mycelial growth of *Centrospora* was inhibited at a concentration of about $^1{}_2$–1 p.p.m. of Hg, $^1{}_2$–1 p.p.m. of hydroxyquinoline sulphate and 8 p.p.m. of blue vitriol.

B. *Experiments with chemical treatment of the soil*

In two preliminary experiments carried out in March 1948 3 groups of 10 plants were watered with blue vitriol in concentrations of 0.05, 0.1 and 0.2 per cent respectively, 4 groups of 5 plants were watered with Germisan in concentrations of 0.025, 0.05, 0.1 and 0.2 per cent respectively, and 4 groups of 5 plants watered with the above mentioned Agranin in the same 4 concentrations. None of the plants were damaged by these treatments.

On December 3, 1948, that is about one month before the first outbreak of the disease would normally be expected, potted plants were placed in frames heated by steam pipes and watered with various concentrations of hydroxyquinoline sulphate and Germisan. In each frame 120 plants were placed. 3 groups of 120 plants in 3 frames, placed as far as possible from each other, were treated with each chemical and concentration (12 *l* of liquid was used for each group of 120 plants). $23^1{}_2$ frames, distributed equally among the treated lots, and containing altogether 23.5 × 120 plants, remained untreated. Table III gives the results recorded on February 4, 1949.

On account of the negative result of this experiment it was decided to try the effect of chemical treatment carried out at an earlier stage of growth. This new experiment with chemical treatment of the soil was combined with an experiment on steam treatment of the soil, the latter being dealt with later. Table IV records the results obtained from this combined experiment.

The plants were treated twice with the fungicides, the 1st time in July 1949, 8 days after the seedlings had been pricked out, the 2nd

TABLE III

Experiment on chemical treatment of the soil as a control measure against *Centrospora acerina* on *Primula malacoides*. Treatments on December 3, 1948, results recorded on February 4, 1949

Number of plants	Chemical	Concentration per cent	Number of dis. plants	Percentage infected
3 × 120	Hydroxyquinoline	0.01	71	19.7
3 × 120	sulphate	0.05	64	18.0
3 × 120		0.1	74	20.6
3 × 120	Germisan	0.01	63	17.5
3 × 120		0.05	66	18.5
3 × 120		0.1	45	12.5
23.5 × 120	Untreated		541	18.5

TABLE IV

Experiment with chemical and steam treatment of the soil as control measures against *Centrospora acerina* on *Primula malacoides*. For conditions of treatment, see the text. Results recorded on February 28, 1950

Steam treatment. All plants potted in sterilized soil	Chemical	Concentration per cent	Number of plants treated	Number of plants infected	Percentage infected	
Plants pricked out in unsterilized soil	Blue vitr.	0.2	200	13	6.5	
	Hydroxyquin. s.	0.1	200	16	8.0	
	Agranin, Folosan	0.2	187	16	9.1	average
	300 g pr m³ soil		200	20	10.0	*7.0*
	untreated		200	3	1.5	
Plants pricked out in *steam sterilized* soil	Blue vitr.	0.2	200	0	0	
	Hydroxyquin. s.	0.1	200	2	1.0	
	Agranin, Folosan	0.2	200	1	0.5	average
	300 g pr m³ soil		200	1	0.5	*0.6*
	untreated		183	2	1.1	

time when the potted plants were moved on October 10 from cold frames to heated frames. An exception was the plants treated with Folosan. They were treated only once that is when the plants were potted, the powder being mixed with the soil.

It appears that the chemical treatments at the concentrations used did not give any control of the disease.

C. *Experiments with steam treatment of the soil*

In 1949–50 and 1950–51 experiments on the effect of disinfection of the soil by steaming were carried out. In both years the soil was

treated in April, each ingredient to be used in the soil mixture (peat, farm yard manure, loam, and sand) being treated separately. A temperature of nearly 100°C was maintained in the soil for a few minutes. The seed was sown, and the resulting seedlings were pricked out and later potted (in new pots) in treated soil.

The results of the 1949–50 experiment are recorded in Table IV. In this experiment plants from seedlings pricked out in unsterilized soil, but potted in sterilized soil, served as controls. When all the groups, whether treated with a fungicide or not, are taken into consideration (the average infection rate being 7.0% for untreated and 0.6% for treated) there seems to be no doubt about the effectiveness of steam disinfection in preventing development of the disease.

Further evidence that steam treatment is an effective procedure was obtained in an experiment performed during 1950–51 when the control plants were not only pricked out but also potted in untreated soil. The results recorded on March 28, 1951 showed that, of 285 plants in sterilized soil, 9 plants, *i.e.* about 3 per cent, were diseased, but on examination of the roots of 1 of these plants *Centrospora* could not be found.

Of 400 plants in unsterilized soil 79 were diseased, *i.e.* about 20 per cent. In 5 of 6 of these plants examined for the presence of *Centrospora* the fungus was found in abundance.

Finally, out of about 5400 plants of the same variety cultured in sterilized soil only 3–4 per cent were, in the estimation of the nurseryman, attacked by the disease.

SUMMARY AND CONCLUSIONS

A serious root rot of *Primula malacoides* has caused great damage in at least one nursery in Denmark. In one year about half of the whole crop in this nursery was totally destroyed, and since 1932 the disease has caused annual losses to the grower, amounting to 12.000 to 25.000 kr.

The characteristic resting mycelium of *Centrospora acerina* was consistantly observed in the attacked roots. From such mycelium, and from conidia developed from it, the fungus could easily be isolated.

By inoculation, reisolation and reinoculation the fungus was proved to be pathogenic (Table I and Table II) and to produce the same symptoms as those developed by plants spontaneously attacked.

In diseased plants the main root or a number of the larger roots are always found to be more or less rotten and the resting mycelium is present in abundance in the diseased tissues. Primary rot symptoms on the leaves

and petioles have not been observed. The tops of the plants show the following sequence of symptoms, at first the leaves lose their fresh colour, then they become slightly chlorotic with a muddy greenish tint, later the leaves gradually become softer and wilted until finally the whole plant collapses.

Soil treatment with various chemicals did not give adequate control at the concentrations tested.

Experiments with steam disinfection of the soil in successive years gave good results. In the first a reduction in the incidence of infection, from 7.0% in unsterilized soil to 0.6% in sterilized soil, was obtained, while the second experiment showed a reduction from about 20 per cent in unsterilized soil to about 3 per cent in sterilized soil.

From these results, on the effect of steam soil disinfection it is concluded that the important source of infection is the soil and that suitable steam treatment of the soil is an adequate control measure.

It should be emphasized that diseased and dead plants with their infested root balls should not be thrown on compost heaps but should be thoroughly destroyed. It should also be remembered that the pathogen has proved capable of attacking a great number of hosts belonging to widely different genera.

The fungus is probably not, or at least extremely seldom, seedborne on *Primula malacoides*. It was not found by examination of more than 50 seed lots during the last 5 years.

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THE ROOT SAP OF BIRCH ON A PODZOL

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INTRODUCTION

The effect of birch (*Betula pubescens*) as a soil improver on strongly podzolised soils may not be unconnected with its characteristic rooting habit in such soils. Birch is unusual in being able to penetrate an iron pan at an early age and so exploit unleached soil. This may influence the nutrient balance of the tree as a whole and thereby affect the nutrient status of the birch litter.

As an exploratory enquiry to see if there was any factual evidence for this hypothesis, sap was collected in May, June and July, 1950, from the severed roots of birch trees growing on the North Yorkshire Moors, and analyses carried out for the more important nutrients. The roots were than excavated and their distribution relative to the soil horizons described. Attempts were also made to collect sap from pine for comparative purposes, but pine proved a much more difficult subject than birch and the results are quite inadequate for any detailed comparison. In fact, even the birch data are so variable that only the broadest conclusions may be drawn. The fact that a new tree has to be used for each sampling precludes any standardisation of conditions, and site differences, soil heterogeneity and the variability of the specimens make for large errors. Even in any one sampling the great differences in the vigour, distribution and extent of the various roots all make for large variation.

FIELD TECHNIQUE

The birches selected were as far as possible of the same age and size, namely about 12 years old and 6 to 8 ft. high, growing solitary

amongst dense heather (fig. 1); coppiced trees were avoided. The pines selected were vigorous specimens of about the same age as the birch.

The first operation was to locate the surface roots radiating from the stock and to cut them, marking the positions of the cut ends with stakes. It was found most convenient to cut them about $1\frac{1}{2}$–3 ft. from the base of the tree. This being done, any sinkers under the root stock were also cut and the aerial part of the tree removed.

Each cut root then had the soil dug away from beneath it so that it could be bent downwards. The cut surface was pared with a clean razor and the root sealed into the neck of a 250 cc conical flask with tightly packed cotton-wool. The flask contained 1 cc of 10% formaldehyde to prevent any bacterial or fungal growth in the collecting sap. The flask was then pegged down so that the root was pointing slightly downwards, and left until enough sap had collected (fig. 2). In practice this varied from a week, if tapping coincided with a rainy period, to four weeks or even more during the drier parts of the summer.

Stout vertical roots occasionally resisted attempts to bow them down to the necessary position, but careful excavation of the soil around the upper part enabled all but the thickest of such roots to be tapped successfully. It might be thought that the cotton-wool plug is an inadequate protection against rain, but such seals have withstood torrential thunder-storms without leaking; the only occasions on which rain-water has entered the flasks have been when the hollows dug out beneath the deeper roots have become flooded and the water had risen over the top of the necks of the flasks.

When enough sap had been collected the flasks were removed and the roots carefully excavated to the fibrous endings of every branch — no easy matter in stony and compacted soils. The only circumstances in which this was not taken to completion was when a root had penetrated the pan and was ramifying in the subsoil; from the point of view of nutrient uptake it was regarded as unnecessary to study the exact course of such roots once they had reached the C horizon. Particular note was made of such features as the horizon in which the root ran, the presence of sinkers and whether they penetrated the pan, any exceptional amount of fibrous rooting, including mycorrhizal clusters, the penetration of rotting wood, old root channels, etc.

Fig. 1. Young birch in dense heather.

Fig. 2. Collecting flasks in position on pine roots.

Fig. 4. Part of the surface root system of a birch.

ANALYTICAL METHODS

The analysis of the sap was carried out by the following methods:

Calcium, Potassium, Sodium — by the flame photometer.

Phosphorus — colorimetrically as the molybdenum blue complex, using a "Spekker" photometer.

Magnesium — colorimetrically with Titan Yellow. Preliminary tests showed that the calcium and phosphate concentrations were too small to interfere, as were also those of other metals such as manganese, iron and aluminium.

Manganese — normally by oxidation to permanganate with periodate; but where very small quantities were shown to be present by this method, the more sensitive tetramethyldiaminodiphenylmethane method of Cornfield and Pollard[1]) was used as a check.

Iron — colorimetrically with potassium thiocyanate.

Ammoniacal Nitrogen — by distillation with alkali, followed by an acid-alkali titration.

pH — electrometrically with a glass electrode.

Note: Tests for nitrate never showed more than a trace, so were not included in the standard procedure.

INTERPRETATIONS OF RESULTS

The results shown in fig. 3 and the tables may be considered in conjunction with those of a similar investigation by Ladefoged[2]) on birch (species unspecified) growing on a brown forest soil in association with oak. He found that two factors governed the uptake of nutrients, namely the water content of the soil and the seasonal change in the content of available nutrients in the soil. Thus he found that potassium and nitrogen (and probably calcium) were absorbed in greater concentrations from wetter soil, whilst phosphate appeared to be absorbed independently of the percentage of water in the soil. However, towards the end of June, even at the same soil moisture percentage, the concentrations of most nutrients absorbed were reduced.

In fig. 3 the present results have been arranged in ascending order of the amount of sap produced by the respective roots. It has already been pointed out that the production of sap is related to rainfall, so that one may fairly suppose that a root producing much sap is in a wetter local environment than an inactive one. If Ladefoged's observations are applicable to the present conditions, one would therefore expect to find the concentration of nutrients in-

creasing with the amount of sap. Though the variation is too great to allow conclusions to be put forward as statistically significant,

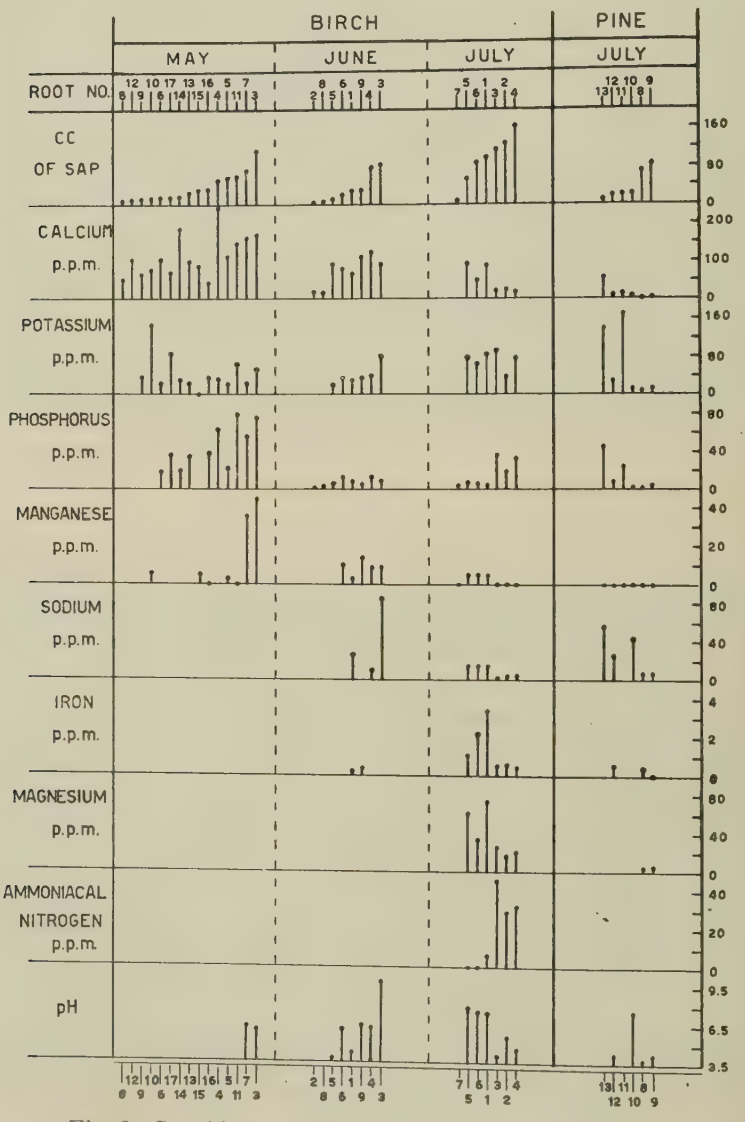

Fig. 3. Graphical representation of sap analyses, 1950.

there does seem to be an upward trend in the concentrations of calcium and phosphorus as the amount of sap increases during the

months of May and June; potassium, on the other hand, shows no apparent correlation. In this there is a contrast with L a d e f o g e d's findings in that he found potassium to have a strong connection with water content of the soil, whereas phosphate seemed to be independent.

During May and June these relationships hold irrespective of the type of roots producing the sap or indeed of their distribution through the soil horizons. But in July a marked contrast is to be seen. The tree studied in this month happened to have a root system clearly defined into three roots which were entirely superficial (nos. 2, 3 and 4) and three which were simple deep roots penetrating the pan (nos. 5, 6 and 1): a seventh root of mixed distribution gave too little sap to allow useful analysis. With the exception of potassium, all the analyses made on the sap of the six vigorous roots showed a definite correlation with the rooting layer; though there was ample water in all horizons, as shown by the large quantities of sap produced, there was no longer such a marked connection between the amount of sap and the concentration of most of the nutrients. Once more potassium is exceptional in that whilst it still remains independent of the amount of sap, it also appears to bear no relation to the depth of rooting. Apart from this, in comparison with the shallow roots, the deep roots despite a lower quantity of sap, have a higher concentration of all the bases (i.e. calcium, magnesium, sodium, iron, manganese) and consequently a much higher pH; the shallow roots, on the other hand, are richer in phosphorus and ammoniacal nitrogen. The averages (parts per million) for each group of three roots illustrate this point (Table I).

TABLE I

Concentration of nutrients in sap from deep and shallow roots of birch (p.p.m.) — July, 1950

Nutrient	Deep Roots	Shallow Roots
Ca	77	25
Mg	55	21
Na	14	4
K	75	70
Fe	2.2	0.5
Mn	5	0
P	7	30
N (ammoniacal)	2	36
pH	7.56	4.87

The high pH values which may be reached in some of these saps is noteworthy in view of the extreme acidity of the soils in which these trees are growing; the subsoil seldom has a pH as high as 5, whilst the surface is about 3.1. The phenomenal value of 9.94 reached by the sap of one root in June may be exceptional, but values between 7 and 8 appear to be normal for the deep roots.

It is impossible to draw any safe conclusions about the seasonal uptake of nutrients on the basis of only three sets of observations, each on a different tree. Averages of the concentrations of calcium, potassium, phosphorus and manganese for all roots are given for each three, month by month, in Table II.

TABLE II

Seasonal variation in average concentration of nutrients in birch root sap (p.p.m.) — 1950

Nutrient	May	June	July
Ca	114	77	51
K	45	41	72
P	45	8	16
Mn	13	9	2

From these one notes tentatively the decline in the concentration of calcium, whilst phosphorus and manganese also appear to be absorbed to a greater extent in the spring. Potassium, once more out of accord with L a d e f o g e d's findings, is taken up to the greatest extent in July.

It is not possible to compare sap yield month by month since production is so irregular and cannot be put upon a proportional time basis. The rate of production of sap is considerably slower than in L a d e f o g e d's birches; even under wet conditions tapping for only 12 to 36 hours would not have produced anything like the quantities that he got; perhaps a week would be a comparable time for similar quantities. It should be made clear, however, that since this work was carried out over 200 miles from the base laboratories in Oxford, it was not possible to keep a close watch on the course of sap production. The collection was normally started on one visit to the area, and on the receipt of a report from an unskilled observer that enough sap had collected, another visit was made to complete the collection and root excavation.

Small quantities of sap can be collected as early as the end of

March, but this comes only from the surface roots. The deep roots do not come into production until about a month later, though the surface roots still remain the greatest producers. Some preliminary tappings were made during 1949, which turned out to be a particularly dry summer, and though inadequate detailed records were kept of the root distribution there was a marked change during June of the main source of sap. At the beginning of the month surface roots were still the main producers, although at a lower rate than in the same month of 1950 which was a much wetter year. By the end of the month, however, the surface roots were producing no sap at all, but considerable quantities were coming from the deep roots. This was a striking demonstration of the value of deep rooting in a habitat liable to summer drought. In the wetter summer months of 1950 there was not the same change; even when the deep roots were yielding considerable amounts, as much or more was still being produced by the surface roots.

A further comparison which may be made with Ladefoged's figures is of the actual concentrations of certain nutrients. Averaging out his figures for each month from Table II of his paper, including all depths, estimates roughly comparable with those obtained from the present enquiry are obtained, and these are shown in Table III.

TABLE III

Comparison between root sap analyses on podzol and on brown forest soil (p.p.m.)

Month	Nutrient	Brown Forest Soil	Podzol
May	K	141	45
	P	84	45
	Ca	72	114
June	K	56	41
	P	57	8
	Ca	—	77

It is seen that the phosphorus and potassium contents of the sap from the birch on the brown forest soil (Ladefoged) are markedly higher than those from a tree on the moorland soil. Calcium surprisingly runs at a higher average concentration in the sap of the moorland birch, but Ladefoged's figures only allow comparison for one month.

Ladefoged also made determinations of total nitrogen (organic nitrogen + ammonia) and found an overall decrease in this as the season advanced. His highest values (28 May) averaged 128 p.p.m. for the whole root system. In the present enquiry no systematic determination of total nitrogen was made since it is not necessarily completely dependent upon supply from the soil, but could be affected by translocation from other parts of the plant before root severance. However, some sample estimates were made upon some of the saps collected during May 1950; the values obtained are shown in Table IV.

TABLE IV

Variation in total nitrogen (p.p.m.) in the root sap of a single birch tree — May 1950

Root No.	Total N	Root No.	Total N
3	419	11	443
4	440	12	131
5	122	14	22
6	52	16	193
7	208	17	392

Average — 242 p.p.m.

It is certainly remarkable that the sap of the moorland birch should contain nearly twice as much nitrogen as that of the woodland birch, especially as tree crops on the moor soils frequently show nitrogen deficiency. It may also be noted that the single set of ammoniacal nitrogen determinations gave some values much higher than anything recorded by Ladefoged. However, with an element of such complex function as nitrogen, it may be that long periods of abnormal nutrition inflicted by the prolonged tapping have resulted in physiological changes in the root tissues, and these may in turn be reflected in anomalous nitrogen contents in the sap.

ROOT FORM AND ROOT ENVIRONMENT

In the course of the root excavations, a variety of root forms was encountered. The lengths of the roots varied from a few feet to as much as 6 yards, and there were likewise considerable differences in diameter. The long surface roots were usually relatively simple with few fibrous rootlets (fig. 4); others were copiously branched and had many fibrous endings. The mycorrhizal habit was recorded when

observed, but the majority of rootlets did not show it. Careful record of all these variations was kept, but no consistent correlation could be found between root form and either the yield of sap or its composition. One may conclude that local heterogeneity in these soils is sufficiently great to mask all other correlations.

In a similar way, the environment of the root, as assessed by the naked eye, failed to show any correlation with quantity or composition of the sap, apart from the broad distinction between roots which penetrated to the subsoil and those which did not, as already described. It was not uncommon to find roots entering a rotten stump or root, the remains of an ancient pine plantation, and when they did so they often developed large clusters of mycorrhizal rootlets in the rotten wood. Even this striking difference in feeding habit was not reflected in the analyses, but it must be emphasised that no estimates of organic nutrients were made.

In one or two cases there appeared to be some connection between the amount of sap produced and the density of the heather; in dense heather the yield was usually small, but where the roots exploited an open patch, more sap was produced. This was probably only true at certain seasons, when competition for water between the surface roots of birch and heather was most severe.

SAP FROM PINE ROOTS

As mentioned earlier, the flow of sap from pine roots is much less regular than from birch. In 1950 it was found that pine did not start producing any sap until June, and even then it was not vigorous. In 1949, on the other hand, a heavy yield was obtained in June, although the year was drier. The unpredictability of pine yield is well illustrated in Table V.

TABLE V

Yield of sap from pine roots — cc		
Year	Month	Yield per tree
1949	Mar/Apr.	Nil
	June	592
	July	Nil
1950	May	Nil
	June	5
	July	225

On account of this variability no seasonal comparisons can be made. No analyses are available for the June 1949 sap, and the only test carried out on the June 1950 sap showed a total absence of manganese, in which at least it was consistent with the analyses for July.

The only comprehensive results are those for July 1950, which are also represented in fig. 3. There does not appear to be any correlation between the quantity of sap and the concentration of any nutrient, but there does seem to be some parallelism in the variation of the respective quantities of calcium, phosphorus and to a lesser extent potassium. The pH of the sap lies in the region of 4, with the exception of that from one root which has a pH of 7.6, probably a result of its unusually high sodium content.

Table VI gives the data for comparison between the averages of these analyses and those for birch at the same time (Table I).

TABLE VI

Concentration of nutrients in sap from shallow roots of pine (p.p.m.) — July 1950	
Nutrient	Concentration
Ca	15
Mg	8
Na	29
K	63
Fe	0.3
Mn	0
P	14
pH	5.04

With the single exception of sodium the nutrient status of pine sap is below that of even the shallow roots of birch. At this time of the year the pine tree on these moorlands must be receiving a smaller quantity of mineral nutrients than the birch growing under identical conditions. Its root system is generally less extensive than that of birch.

Though it has been shown already that the birch is absorbing nutrients very vigorously for over a month before the pine starts, there is no information as to how long the process continues into the autumn in each species. But it seems likely that over the year as a whole the birch absorbs considerably greater quantities of

minerals from the soil, which is possibly contributory to the generally higher mineral content of birch litter as compared with pine litter. This must be a factor of considerable importance in the favour of birch as a soil improver, especially as birch litter is more freely broken down than that of pine.

CONCLUSION

Imperfect though this inquiry is, it has confirmed that the unique rooting habit of birch in these soils may confer upon it great advantages as a soil improver and as a pioneer species. In being able to reach the subsoil, not only does it absorb greater quantities of mineral nutrients, it also can and does tap a more permanent water supply to tide it over periods of surface drought. In comparison with pine, its main rival as a coloniser of moorlands, one notes with interest though perhaps not with surprise, the superior ability of birch to extract nutrients even from the upper layers of the soil. Not only does the morphology of its root system favour its uptake of nutrients, but in the same rooting levels as pine its root physiology enables it likewise to be more effective.

Finally may be noted the change from the high concentration of nutrients being translocated by birch roots in the spring, to lower concentrations (in most cases) as the summer progresses. This is presumably parallel to a similar diminution observed by L a d e f o g e d and reasonably attributed by him to the progressive depletion of these nutrients in the upper layers of the soil. This is doubtless due not only to the activity of the birch but of the other competing vegetation as well, combined with a reduction in microbiological activity as the soils dry out.

The results of the analyses carried out in 1950 are given in full in Table VII.

TABLE VII

Complete analyses for birch and pine, 1950
Analyses — p.p.m.

Month 1950	Root No.	Duration of Tapping Days	Sap Yield (cc)	Ca	K	P	Mn	Na	Fe	Mg	N(NH_4)	N (Total)	pH	Soil Horizons
Birch														
May	3	35	115	170	53	76	45	—	—	—	—	419	5.94	A
	4	35	50	240	31	62	—	—	—	—	—	440	—	A
	5	35	56	110	25	22	3	—	—	—	—	122	—	A
	6	35	15	102	25	19	—	—	—	—	—	52	—	A
	7	35	69	159	25	55	36	—	—	—	—	208	6.20	C
	8	35	4	50	—	—	—	—	—	—	—	—	—	old stump
	9	35	8	66	37	—	—	—	—	—	—	—	—	A.B.C.
	10	35	14	76	147	—	6	—	—	—	—	—	—	old stump
	11	35	59	147	63	80	0	—	—	—	—	443	—	A.B.
	12	35	6	102	—	—	—	—	—	—	—	131	—	A
	13	35	23	100	25	35	—	—	—	—	—	—	—	A.B.C.
	14	35	16	186	31	20	—	—	—	—	—	22	—	B.C.
	15	35	30	87	0	—	5	—	—	—	—	—	—	B.C.
	16	35	32	47	37	39	0	—	—	—	—	193	—	B.C.
	17	35	15	72	83	37	—	—	—	—	—	392	—	B.C.
June	1	30	27	72	29	10	3	27	—	—	—	—	4.25	A.C.
	2	30	2	23	—	1	—	—	—	—	—	—	—	A.B.C.
	3	30	82	92	80	9	9	86	0.3	—	—	—	9.94	A.B.C.
	4	30	78	125	40	13	9	10	0.2	—	—	—	6.30	A.C.
	5	30	11	94	22	6	—	—	—	—	—	—	3.74	A.C.
	6	30	20	78	38	13	11	—	—	—	—	—	5.88	B.C.
	8	30	3	20	—	3	—	—	—	—	—	—	—	A
	9	30	28	110	35	6	14	—	—	—	—	—	6.40	B.C.
July	1	12	100	90	84	4	5	14	3.4	72	6	—	7.44	B.C.
	2	17	128	27	40	20	0	4	0.6	17	29	—	5.60	A
	3	20	117	24	92	38	0	2	0.5	26	46	—	4.15	A
	4	20	162	23	79	33	0	5	0.4	20	32	—	4.76	A
	5	20	54	92	79	9	5	14	1.05	61	0	—	7.85	C
	6	20	86	50	62	7	5	14	2.15	32	0	—	7.40	C
	7	20	7	—	—	4	0	—	—	—	—	—	—	A.C.
Pine														
July	8	28	70	1	9	1	0	8	0.4	7	—	—	3.87	A
	9	28	83	3	16	3	0	8	0	8	—	—	4.32	B
	10	28	22	7	15	2	0	43	—	—	—	—	7.64	A
	11	28	20	17	170	25	0	—	—	—	—	—	—	A.B.
	12	28	19	8	29	9	0	27	0.6	—	—	—	4.34	—
	13	28	11	55	140	46	0	57	—	—	—	—	—	A

SUMMARY

Root sap analyses were made on birch trees growing in a strongly podzolised heath soil. In the early summer the concentration of calcium and phosphorus appears to vary with the quantity of sap produced, and is independent of root environment. Later in the summer, however, this relationship no longer holds; there now being a connection between rooting zone and nutrient concentration. Roots reaching the *C* horizon absorb greater quantities of bases, whilst the superficial roots show higher concentrations of phosphorus and ammoniacal nitrogen. Throughout the season potassium uptake remains practically independent of both sap yield and rooting zone. In these soils young pine trees do not penetrate to the subsoil, but a comparison of sap analyses from surface roots of pine and birch shows that birch sap is richer in most nutrients. This, together with the ability of the birch to exploit the subsoil, may be a contributory factor to the soil improving properties of a birch crop.

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ON THE EFFECT OF STORAGE AND TEMPERATURE ON THE EXCHANGEABLE MANGANESE IN SOIL SAMPLES

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INTRODUCTION

Many soils do not contain sufficient available manganese for normal growth of plants. Soil scientists have therefore been interested in determining the content of exchangeable manganese in soil, for this fraction of the manganese content is considered to be of great importance for the manganese supply of the plants.

No matter what displacing solution is used, it is essential that the fraction of manganese obtained should not be changed during the treatment to which the soil samples are subjected between sampling to analysis. Such changes would make a comparison of the amounts of exchangeable manganese found in the soil at different places or at different times, difficult i.e. the influences of the seasons and other factors would be difficult to evaluate.

As is well-known, it is difficult to analyse moist soil as sampled in field conditions, particularly if there is a large number of soil samples to be analysed. Oven-drying of soil samples produces a very great increase in the content of exchangeable manganese, hence air-dried soil samples are used in most places. But as shown in what follows, it is not sufficient to air-dry the soil to avoid changes in the content of exchangeable manganese during the storage of the soil samples. Thus in 1936 it was observed — as mentioned in the Congress Report of the Scandinavian Society of Soil Science in 1947 (p. 731) — that the content of exchangeable manganese in the soil increased in air-dried soil samples when they were stored.

LITERATURE

In 1913 Kelley and McGeorge [3]) found that the content of water-soluble manganese increased considerably from the moist to the air-dry state in two samples of loam.

McCool [5]) determined the content of water-soluble manganese in three moist soils stored at 35°, 72°, and 100° F respectively (i.e. about 2°, 22°, and 38°C) for 3, 15 and 30 days. He found that the content of water-soluble manganese did not change materially when the samples were stored at 35°, whereas there was an appreciable increase at the temperatures 72° and 100°F, an increase which grew with the length of the storage period.

Fujimoto and Sherman [1]), who examined 28 soil samples, found that the content of exchangeable manganese increased considerably in 22 soils from a moist to an air-dried state. In five soils the content of exchangeable manganese decreased, and in one soil, which had no exchangeable manganese in the moist state, there was no manganese, either, when the soil sample was air-dried at about 27°C.

Heintze [2]) by extraction of soil with $Ca(NO_3)_2$ observed that "the exchangeable form of manganese sometimes increased considerably in soils stored for some years in the air-dry state".

Some results are given below of investigations of changes in the content of exchangeable manganese in air-dry soil samples stored at different temperatures for different periods.

METHODS USED

Steenbjerg's method [7]) was used for the determination of the content of exchangeable manganese in the soil samples. 100 g air-dry soil were extracted with 1 mol $Mg(NO_3)_2$. After addition of HNO_3 and $AgNO_3$ to the soil extracts, the manganese was oxidized by means of $K_2S_2O_8$ into the permanganate ion, and measured colorimetrically. The total amount of manganese found in the soil was computed, and is expressed as the manganese value, T_{Mn}. A T_{Mn} unit = 1 mg Mn per kg air-dry soil = 1 p.p.m.

By air-dry soil samples are meant soil samples spread in a layer of a thickness of at most 1 cm and dried for at most 48 hours at about 20°C.

RESULTS

The relation between the content of exchangable manganese in soil samples in field conditions and in the air-dry state has been investigated here only in the case of three soil samples. The manganese value, T_{Mn}, was determined in the soil samples immediately after sampling and again after air-drying.

The results of the analyses of the three soil samples calculated on an air-dried basis are given in Table I.

TABLE I

Manganese values in moist and air-dry soils			
No.	Soil	Mn, p.p.m.	
		Moist	Air-dry
10	Loam	0	0
11	Sandy soil	0	0.3
12	Sandy soil	2.8	3.1

In these three soils the increase in the manganese value as a consequence of air-drying is of no practical importance.

Next the influence of the period of storage on the manganese value when the air-dried soil samples were stored in tins at about 20°C was investigated. Two soils of light loam in good cultivation, No. 1 and No. 2, were sampled. In Sample No. 1, which was taken from a lawn, the pH was 6.9. In Sample No. 2, taken from a vegetable garden, the pH was 7.0. The manganese values were determined at intervals, which are shown in Table II.

TABLE II

Manganese values in loam after different periods of storage			
Date of analysis	Stored for days	Mn, p.p.m.	
		No. 1	No. 2
24/10 1936	2	2.0	1.8
5/1 1937	75	4.7	4.2
13/11 1937	387	6.6	7.1
23/2 1938	489	8.4	7.9
12/10 1938	718	7.5	6.6

As appears from Table II, the increase of the manganese value in these soils does not cease until well over a year.

A similar investigation was made of two sandy soils, No. 3 and No. 4, taken from diluvial sand at Ulstrup, Jutland, where the crops suffered from deficiency of manganese. The results are given in Table III.

TABLE III

Manganese values in sandy soil after different periods of storage					
Date of analysis	Stored for months	No. 3		No. 4	
		Mn, p.p.m.	pH	Mn, p.p.m.	pH
2/8 1938	—	0.9	7.2	0.8	7.2
2/9 1938	1	1.7	—	1.6	—
2/2 1939	6	3.1	7.0	3.2	7.1

Here, too, the manganese value increased considerably during the storage of the soil samples.

Then two soils were examined which were sampled from a liming experiment in the marsh of Ribe, a very heavy loam which is also rich in humus.

TABLE IV

Manganese values in very heavy loam after different periods of storage					
Date of analysis	Period of storage	No. 5		No. 6	
		Mn, p.p.m.	pH	Mn, p.p.m.	pH
28/11 1938	—	15.4	6.5	2.8	8.1
9/1 1939	6 weeks	26.7	6.5	—	7.9
10/6 1939	$6^1/_2$ months	34.8	6.0	11.4	7.9

The contents of exchangeable manganese also increased greatly in both these heavy loams with rather different pH values.

Next, an attempt was made to show the influence of temperature on the increase of the manganese value when the soil samples were stored for a shorter or longer period.

In Sample No. 10 (see Table I) the manganese value was 0 in the moist soil, in the soil air-dried at 20°C there was so little manganese that it could not be measured by the methods used. From this sample three portions of moist soil each weighing 115 g, corresponding, to 100 g air-dry soil, were then dried in a thermostat for 4 hours at 50°, 100°, and 150°C respectively, after which the manganese value was determined. The results are given in Fig. 1.

When this loam poor in manganese is heated for 4 hours, the content of exchangeable manganese increases greatly with increasing temperature.

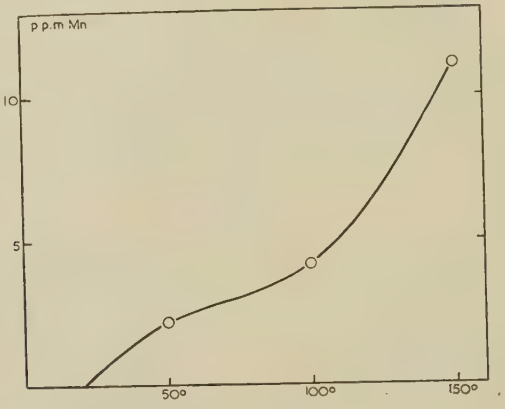

Fig. 1. Soil No. 10 stored for four hours at different temperatures.

In continuation of this, the influence of temperature on the content of exchangeable manganese when air-dried soil samples were stored for a prolonged period was examined. Two soils, No. 7 and No. 8, were sampled, from a liming experiment on loamy soil at The State Experiment Station, Lyngby, and one sample of sandy soil, No. 9, from the field of Lyngby Agricultural School. In these three soil samples manganese values and pH were determined after air-drying at about 20°C for 48 hours. Then each of the samples was divided into three portions for storage at different temperatures for different periods. Set I was stored in a basement where the temperature fluctuated between 3° and 10°C. Set II was kept in the laboratory at 20°–22°C. Set III was stored in a hot-air oven in which the temperature for 8 hours out of each 24 hours was at a maximum of 60°–70°C. In the case of Sets I and III conditions did not allow storage at a constantly low and a constantly high temperature respectively, but the investigation was at any rate made at three different levels of temperature.

Manganese values and pH of the soil samples were determined after 1 month, 6 months, and 1 year respectively. The results appear in Table V.

TABLE V

Manganese values and pH in air-dried soil samples stored at different temperatures for different periods

Date of analysis	Sample no. 7						Sample No. 8						Sample no. 9					
	Soil		Mn, p.p.m.		pH		Soil		Mn, p.p.m.		pH		Soil		Mn, p.p.m.		pH	
11/6 1938	Loam		5.6		4.7		Loam		0.9		6.5		Sandy soil		1.5		6.3	
	I		II		III		I		II		III		I		II		III	
	Mn, p.p.m.	pH	Mn, p.p.m.	pH	Mn, p.p.m.	pH	Mn, p.p.m.	pH	Mn, p.p.m.	pH	Mn, p.p.m.	pH	Mn, p.p.m.	pH	Mn, p.p.m.	pH	Mn, p.p.m.	pH
12/7 1938	9.0	4.8	10.0	4.9	13.8	4.4	1.6	6.7	1.6	6.6	4.5	6.0	3.0	6.2	4.0	6.3	11.0	5.9
13/12 1938	8.5	4.8	7.9	4.6	13.0	4.3	2.2	6.6	2.7	6.3	5.3	6.0	4.5	6.1	6.4	6.1	12.5	5.7
13/6 1939	17.9	4.7	17.9	4.8	21.7	4.2	4.8	6.5	3.4	6.2	8.7	5.9	14.3	6.0	26.3	6.0	32.3	[illegible]

In all the three soils and at all levels of temperature considerable increases in the manganese values were found after a month, the highest, however, in Set III. During the following 5 months the manganese value in the loam with the low pH, No. 7, decreased for all the three levels of temperature, while it increased in soils No. 8 and 9. During the second period of 6 months the manganese value increased everywhere, as a rule considerably more than during the first six months.

Furthermore it appears from Table V that the manganese values of air-dried soil samples besides depending on the length of the storage period also depend on the storage temperature. The increase as a consequence of the storage temperature is most pronounced with temperatures from 20–22° to 60–70°C. In the case of all storage periods and the two loamy soils there was no great difference between the quantities of exchangeable manganese whether the soils were stored at 3–10° or at 20–22°C, while the manganese value in the sandy soil after 1 month's storage increased by about 33 per cent, after 6 months by about 42 per cent, and after 1 year by about 84 per cent.

The pH values in soil No. 7 regardless of the length of the storage period are of the same order of magnitude within Sets I and II. In Set III there is a distinct fall in the pH as compared with the value measured on June 11. In soil No. 8 pH does not change in Set I with time, whereas there is a slight decrease in Set II and a somewhat greater decrease in Set III. In the sandy soil pH decreases with the increase of the storage period at all levels of temperature.

With increasing temperature there is as a rule a slight fall in pH.

For reasons of laboratory technique one soil sample was investigated to determine whether the manganese value would change when the sample was stored undried until the date of analysis. It was keft in the cardboard box generally used for the sending of samples (i.e. actually a slow air-drying in a cardboard box).

The boxes were stored at two levels of temperature, in the basement, where the temperature during the period of storage varied from 3 to 10°C, and in a thermostat at 22°C.

A large sample of light loamy soil was taken. 115 g soil, corresponding to 100 g air-dry soil, was air-dried for 24 hours, after which the manganese value was determined to be 2.6 and the pH to be 6.7. Simultaneously the rest of the large soil sample was divided into 26

samples of 115 g moist soil, which were stored in close-fitting cardboard boxes with a base of 64 cm^2 and a height of 5 cm. One half of the samples were stored in the thermostat and the other in the basement until the analysis was made, when one sample from the thermostat and one from the basement were analysed simultaneously after they had been further air-dried at about 20°C spread in a thin layer on paper for 2–6 hours. The soil samples were extracted immediately after this last air-drying.

The first set of soil samples were analysed on April 5th. The results appear from fig. 2.

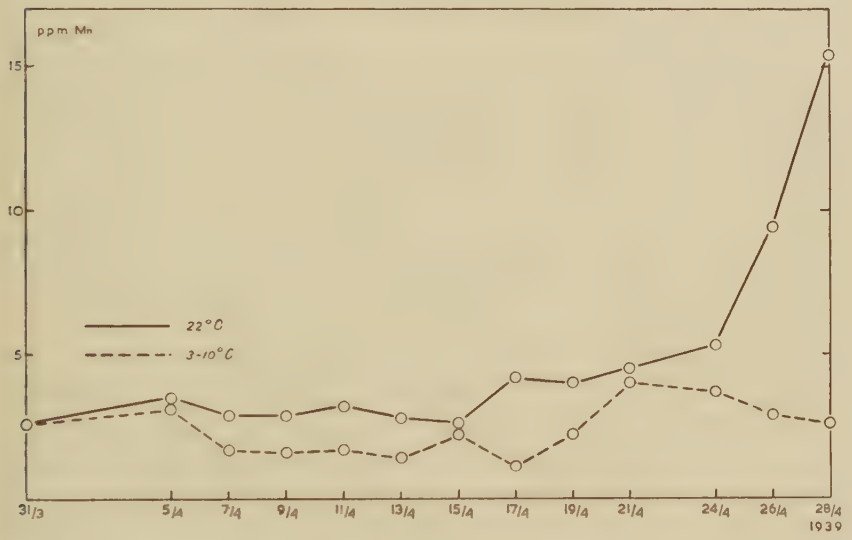

Fig. 2. Soil No. 13 stored in cardboard boxes at different temperature levels.

The manganese values in the samples stored at 3–10°C are somewhat lower than the manganese values in soil samples stored in the thermostat at 22°C, as might be expected from what has been stated above. During the 10 days from the 5th to the 15th of April there were no great changes in the manganese values at either of the two levels of temperature. After April 15th the manganese values increased appreciably in the soil samples stored at 22°C, while the manganese value in a soil sample stored at 3–10°C until April 28th remained at 2.6. The fluctuations in the manganese values of the soil samples stored in the basement were somewhat greater than

those of soil samples stored at 22°C. This was presumably due to the fact that it was possible to maintain a constant temperature in the thermostat, while in the basement the temperature fluctuated between 3° and 10°C.

On Aug. 31st, 5 months after the sampling, the manganese value in a soil sample stored in the thermostat was 23.3 and in a soil sample stored in the basement 10.0.

On April 15th pH in the soil samples at both temperatures was still 6.7, while on April 28th it had decreased to 6.6.

DISCUSSION AND CONCLUSION

The comparisons of the amounts of exchangeable manganese (T_{Mn}) in the moist and in the air-dry state are only based on three soil samples (Table I). It was shown that the increase in the manganese value as a consequence of air-drying was of no practical importance. These results apparently do not coincide with those found by Fujimoto and Sherman[1]); they found a considerable increase in the content of exchangeable manganese in 22 out of the 28 soil samples examined. The explanation of this discrepancy may be that the soils from Hawaii were air-dried for some weeks at about 27°C, while the air-drying here covered at most 48 hours at 20°–22°C.

When a sample of moist loam is heated for 4 hours, the content of exchangeable manganese increases greatly with increasing temperature within the range of temperatures tried: 20°–150°C.

When the soil was extracted with 1 mol $Mg(NO_3)_2$ investigations of 9 different soil samples (Tables II, III, IV and V), including sandy soil, loam, and very heavy loam, showed that the amount of exchangeable manganese increased in air-dried soil samples when these are stored at room temperature or at higher temperatures. The increase which is of considerable magnitude after a month, is greater when the storage temperature is higher. The increase of the manganese values is a function of both the storage period and the storage temperature.

Simultaneous determinations of manganese values and pH values in the same soil showed as a rule a slight decrease in the pH value when the manganese value increased. This fall indicates that reduction processes may take place in soil samples under the conditions of storage used. It is possible that in Danish soils, which are relatively high in humus content, local reduction processes take place in the soil particles which cause an increase in the amount of exchangeable manganese during storage in an air-dry state. There is a possibility that bacteria and fungi may participate in these processes even in air-dried soils, since Khalil's investigations[4]) show that about 20 per cent of the bacteria are still active in the soil after it has been air-dried down about a 5 per cent water content. It is also

possible, as mentioned by Sherman and Harmer[6]) and by Fujimoto and Sherman[1]) that during drying of soil samples a dehydration of complex manganese oxides takes place causing some manganese to become soluble.

McCool[5]) showed that the content of water-soluble manganese in the soil was highest in the month of August as compared with the months of May, June, July, and September. The increase in the content of exchangeable manganese in air-dried soil samples at increasing temperature as found in the present investigations presumably originates partly from the same causes which operate in the field under natural conditions during the warmest months of summer. The seasonal variation must thus also be considered as an estimate of the amount of soluble manganese available for the plants during the changing seasons.

The increase in the content of exchangeable manganese in air-dried soil samples under storage is of such an order of magnitude that it must be considered when manganese analyses are made. If examination of a large number of soil samples including different soils shows that this increase can be avoided by storage of the samples at a low temperature, the practical solution of the problem will give no particular difficulties.

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FIELD PLOT STUDIES WITH SAWDUST FOR SOIL IMPROVEMENT †)

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The use of wood wastes as sources of soil humus has received increasing attention of late years. This is largely because the usual crop rotations seldom maintain soil humus at the highest desirable level and because the increased mechanization of agriculture has made animal manures less abundant and often more costly. The chief form of wood wastes thus tested has been sawdust, although other forms, such as chips, usually have similar effects.

The literature on such use of sawdust has been quite adequately reviewed by Allison and Anderson [1]). Most investigators have found that fresh sawdust requires the application of liberal amounts of nitrogen to secure good crop growth but that sawdust is often beneficial to some soils, particularly after the initial drain on the nitrogen supply has somewhat lessened. Comparatively little information is available on use of partly decayed or rotted sawdust.

In order to determine something about the potentialities of rotted sawdust as compared to fresh sawdust and to farm manure in improving a poor soil these field plot tests were undertaken.

EXPERIMENTAL METHODS

The plots were located on a level area near the Agricultural Experiment Station at Durham, N.H. The land had not been under cultivation for over 15 years. The soil type was a Merrimac loamy

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coarse sand, and was very uniform throughout the area. Tests showed it to be very low in all of the principal plant nutrients. The soil pH averaged about 5.5. For the first year two groups of plots were used; one containing 17 plots and the other 12 plots. For the remaining period of three years the 17 plot field was not used and the 12 plot field was enlarged to contain 20 plots. The size of each plot was 95 feet by 15 feet, or about $^{1}/_{20}$ acre. The variables tested were four: (1) rotted sawdust, (2) fresh sawdust, (3) manure, (4) control (no organic matter). This allowed a replication of each treatment seven times the first year and five times the other years. The replications were laid out in a randomized block system.

The experiment was started in the spring of 1948. The land was first plowed, then 950 pounds of 10–15–10 complete fertilizer, 350 pounds of magnesium sulfate and 1500 pounds of hydrated lime was spread uniformly over the plot area. The plots were marked out and applications of sawdust and manure were made as follows: In all, 9 truckloads of fresh sawdust (coniferous) weighing 50,130 pounds or approximately 25 tons were applied, and 9 loads of rotted sawdust (coniferous) weighing 65,700 pounds or 32.8 tons. This would amount to an application of 5.6 pounds of fresh sawdust per square foot or 121.9 tons per acre and 6.0 pounds of rotted sawdust per square foot or 130.6 tons per acre. The volumes of these two were nearly equal as indicated by the number of loads but because of greater moisture content the rotted sawdust weighed more. The rotted sawdust was obtained from a large pile that had been standing for 10 years. An effort was made to spread both kinds of sawdust at a uniform depth of 3 inches and to allow a one foot margin at the edge of each plot. Subsequent harvest data were arranged to omit the yields from the border rows so as to avoid the marginal influence on the results.

The manure was spread at a rate of one load per plot, each load weighing approximately 3 tons. Therefore, about 24 tons of manure were applied to 8 plots. The 17 plot field was assigned one extra plot of manure the first year to take care of the uneven number of plots.

The land was harrowed by disk harrow twice; once before and once after spreading the sawdust and manure. Then the fertilizer was spread and the land harrowed with a rigid tooth harrow.

In the spring of the second year when the 12 plot field was enlarged to 20 plots, applications of the sawdust and manure to the additional plots were made at the same rates as above. No other

applications of sawdust or manure were made later, so that the yield data from later crops represent residual effects.

The crop grown was field corn (maize) of the Maine Hybrid-B-Variety. It was planted in rows 3 feet apart, about 7 inches apart in the row, by machine the first year. It was planted in hills by hand 3 feet apart each way each year thereafter. Excess seed was planted and then thinned to 3 plants per hill. This was done because a far more uniform stand could be obtained than by machine.

The first year by mid-July a marked stunting and chlorosis of the plants in the fresh sawdust plots was observed. This indicated nitrogen shortage which was confirmed by soil tests. Shortly after this a side dressing of 400 pounds of sodium nitrate was applied between the rows. Cultivation for weed control was done by tractor-cultivator and some hand hoeing and pulling of weeds was done.

Harvesting was done after full maturity of the plants and consisted of husking and weighing the ears. Soil tests of nutrients remaining in the soil showed nitrogen quite low in all but manure plots which were medium to low in level. All other nutrients were moderately low. Soil pH was slightly higher in most plots.

Fertilizer applications the second year (1949) consisted of magnesium sulfate 500 pounds, complete fertilizer 5–10–10 formula 1500 pounds, hydrated lime 1000 pounds, ammonium nitrate 500 pounds.

The soil nutrient tests at the end of the 1949 season showed very similar results to those of the previous year. For the season of 1950 the following amounts of fertilizer were applied: complete fertilizer 8–16–16 formula 2080 pounds, ground limestone 600 pounds. At the end of the 1950 growing season, the level of nutrients in the soil was found to be high enough to make necessary only a very moderate application of fertilizer for the season of 1951. This consisted of 560 pounds 8–16–16 complete fertilizer and 250 pounds of ammonium nitrate.

EXPERIMENTAL RESULTS

The total yields for the different treatments are presented in bar graph form in Fig. 1. Each section of a bar represents the yield for a given year. In this connection the weather conditions for the different growing seasons deserve some comment. The first summer was moderately dry but the plants grew well. The second season there was a sufficient amount of rainfall for the plants to grow fairly well up

to late in July, although there was almost no rainfall from early in June until some time in September. By mid-August it seemed improbable that the plants would be able to form ears. Pronounced wilting and curling of the leaves was evident. There was no notice-

Fig. 1. Total yields of corn (pounds) from field plots.

able difference in this respect in any of the plots. Therefore, on August 25, samples were taken for yield data on the stalks and foliage. For this purpose 10% of the hills distributed throughout each plot or 36 stalks per plot were cut close to the ground and weighed in the fresh condition. This explains the seeming dispro-

portionate amount of space occupied in the graph for yields for 1949.

The summer of 1950 was again very dry but what little rain fell was spaced in time so that the crop could keep growing and form ears. During this year and the season of 1951 no stunting effect of the fresh sawdust was evident as was visible in the first two seasons. The summer of 1951 was far different than the preceeding three. In fact rainfall was so abundant and frequent that the soil moisture level probably never fell below field capacity to any extent. This is evidenced by the soil moisture determinations to be presented later. In a soil of this type, which was very coarse textured and well drained, such moisture conditions were ideal for good crop growth. This proved to be the case as may be seen from the yields for that year in the graph.

The chief fact that is noteworthy about the yield data is that the yields from the rotted sawdust plots were consistently greater than all the rest for each year, even greater than the manure plot yields. However, in mathematical analysis of the results calculation of the standard error of the difference between means for the rotted sawdust yields compared to the manure yields shows that the difference is not significant. Even when the comparison is made between the rotted sawdust yields and the control yields this is also true, as well as for comparison between yields for individual years. The main reason for this lack of statistical significance is the rather wide variation of the yields for different plots within a given treatment. Part of this in turn, at least for the first year, was due to variations in planting and to crow damage. Other factors contributing to errors were variations in depth of mixing of the sawdust by tillage and blowing of the sawdust by wind from one plot to another (the first year).

Nevertheless, the yields perhaps do indicate a beneficial trend for the rotted sawdust treatment. There are also many beneficial effects from such applications that may not always appear in the yields; such as greater ease of tillage, improved soil moisture relations, etc. Particularly it should be emphasized that weed control was much easier in the sawdust plots than in the others. Not only was cultivation easier, but less weeds grew in them. This was quite noticeable in comparison to the manure plots where large numbers of weeds grew from seeds carried in the manure. This leads to a brief consideration of the soil moisture data.

In the summer of 1949 samples of the soil were taken for gravimetric moisture determinations. These were taken shortly after a rain and again after a long dry period at depths of 3, 6 and 9 inches. For the samples taken directly after a rain both the rotted sawdust and fresh sawdust treated soils showed a definitely higher moisture content than the other two, with the top layer of the fresh sawdust treated soil being highest of all. There were no significant differences in the determinations after a dry period. All the higher levels of soil showed greater moisture amounts than all lower levels in all treatments in the first sampling.

Similar determinations taken during the 1950 season at depths of 6 and 15 inches showed no significant differences in any treatments. The same was true of wilting percentage determinations performed in the greenhouse with sunflower seedlings in samples of soil taken at these same depths.

Information on soil moisture of a different sort was obtained in the growing season of 1951. A Bouyoucos[2]) electrical resistance apparatus was available by mid-season and records were taken during August and September. Two resistance blocks were placed in each plot at a 3 inch depth and readings taken at frequent intervals. As might be expected from the abundant rainfall throughout the season already mentioned, the soil moisture curves for most of the season showed a high moisture content and stayed fairly close together. It is worth noting, however, that the curve for the rotted sawdust did not give as great a moisture indication during the wetter early part of the period as did the other treatments, but in the relatively brief drier periods at the end showed more moisture than the others. This was in marked contrast to the control where the moisture content went down rather abruptly in the dry period. From this it might be concluded that organic materials tend to act as a buffer against rapid fluctuations of moisture in soils. This is in general agreement with results reported elsewhere (3,4).

The final soil nutrient tests made at the end of the 1951 season showed nitrogen very low, phosphorus and potassium averaging medium in amount.

SUMMARY

Heavy application of fresh and rotted sawdust to field plots in comparison to manure and untreated control plots showed no significant difference

in yields of corn over a four year period. The soil type was coarse textured and poor in nutrients at the start. However, the total yields for the rotted sawdust plots were consistently greater each year than those for the other treatments, thus possibly indicating a trend of benefit for the rotted sawdust treatments. Total yields for the fresh sawdust were close to those for the controls.

Heavy applications of fertilizer, especially nitrogen, were necessary the first three years, but only a rather light application was necessary the fourth year.

Soil moisture determinations indicate that all of the organic materials tend to prevent pronounced fluctuations in moisture levels, the rotted sawdust most of all.

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A METHOD FOR DETERMINING IN PEA CULTURES, THE AMOUNT OF MOLECULAR NITROGEN FIXED AND THE AMOUNT OF COMBINED NITROGEN TAKEN UP FROM THE SOIL

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When common varieties of legumes are grown under natural conditions it is difficult or even impossible to determine how much nitrogen is taken up from the soil by their roots and how much is fixed by root nodules. Practically every soil which has been under cultivation for any length of time contains nodule bacteria of common legumes (in northern countries e.g. clover and pea) so that under field conditions it is impossible to produce legumes without nodules. By using as controls non-legumes, which can only utilize combined nitrogen, no reliable picture is obtained of the nitrogen uptake from the soil by the legumes, because of differences in root structure and ability to penetrate the soil, and therefore in their capacity to absorb combined nitrogen from the soil.

Previous observations (1, 2, 3) have shown that the first nodules formed on the roots of peas by an ineffective bacterial strain prevent later infection by an effective strain, at least until an advanced stage of growth. Advantage has been taken of this phenomenon to study the present problem. In principle, seedlings are first inoculated with an *ineffective* strain of pea *Rhizobium* and then planted out when nodulation has well begun. A parallel series is set up adopting the same procedure, but this time using an *effective strain*. The ineffective strain used was H VIII — its ineffectiveness having been confirmed by previous investigations in this laboratory. Thus, using seeds of the same weight (to the nearest milligram, though even then there was some variation in their N content) no nitrogen fixation

could be demonstrated when the peas were inoculated with this strain in sterile cultures. The results of this experiment (Virtanen *et al* [4]) were as follows:

TABLE I

Experiment illustrating the nitrogen fixing capacity of H VIII strain of pea *Rhizobium*. Test plant: Torsdag pea. Substrate: nutrient solution without combined nitrogen. Period of growth: Sept. 9–Oct. 6–11, 1943. Nodules appeared Sept. 17.

Bacterial strain	Dry weight one plant, mg	N of one plant mg
Uninoculated.	308	7.3 ± 0.62
H VIII	306	7.5 ± 0.77
H 8 (effective)	3775	104.5

The average N-content of pea seeds (Torsdag) was ~ 7 mg.

EXPERIMENTAL

Seeds of Torsdag pea (Svalöv) were sterilized by the method used in this laboratory (2) and then allowed to germinate in sterile water contained in test tubes which were pinched at a suitable point to prevent the seeds falling into the water. The seedlings were then transferred to nutrient solutions contained in Erlenmeyer flasks (15.1.1951). Some of these solutions were inoculated with the ineffective strain H VIII, others with the effective strain H 47. In both cases, the first nodules were produced between Jan. 23–25 and on Jan. 29 the seedlings were transferred to open glass jars filled with soil. The soil was a muddy clay with a pH of 6.0 and contained 14.1% organic matter and 0.3% nitrogen. During the course of the experiment, the pots were watered with a nutrient solution containing 60 mg $MgSO_4$, 81 mg KCl, 70 mg KH_2PO_4, 67 mg $CaSO_4$ and 1 ml of 5% $FeCl_3$ solution per litre of tap water. No nitrogen was supplied. The plants were illuminated by a bank of 12 tubular lights. At the end of flowering, the crops from each pot were harvested by cutting at soil level. The soil from each pot was emptied separately onto a sheet of strong paper, the nodules examined and the soil and roots then returned to the pot. On the roots of the peas infected with H VIII, no effective red nodules could be seen, only white ones typical of this strain. On the roots of the peas infected with H 47 however, typical red oblong nodules were present.

Considering that each series consisted of 5 pots each with 4 plants,

the experiment succeeded surprisingly well. In previous experiments with H VIII a bunch of effective nodules were produced on a few occasions after a growth period of $1\frac{1}{2}$ — 2 months.

The experimental results are given in Table II.

TABLE II

Pot experiment with Torsdag pea, seeds sterilized Jan. 15, 1951, seedlings transplanted to pots Jan. 29, crop harvested March 29. Root medium muddy clay, pH 6.0. 4 plants per pot. Artificial light

No. of pot	Bacterial strain	Dry weight, g	N, % of dry matter	Total N of the entire crop
1	H VIII	2.519	3.13	78.75
2	H VIII	2.514	2.97	74.55
3	H VIII	3.466	2.38	82.55
4	H VIII	2.651	2.73	72.45
5	H VIII	2.057	2.56	52.55
	Average	2.641	2.75	72.17
1	H 47	4.517	3.20	144.45
2	H 47	7.252	2.87	208.06
3	H 47	4.035	3.24	130.86
4	H 47	6.837	3.11	212.49
5	H 47	6.121	3.11	190.24
	Average	5.752	3.11	177.22

The amount of N in the above ground parts of each plant derived from the seed itself is approximately 5 mg. The difference between this and the total N content of the peas inoculated with the ineffective strain therefore represents the amount of N actually taken up from the soil and amounts to 52 mg per pot of 4 plants. Assuming that the peas inoculated with the effective strain take up the same amount of N from the soil, then in each pot they have fixed approximately 157–52, i.e. 105 mg of atmospheric N. In other words, the peas inoculated with an effective strain have obtained about 67% of their N from the atmosphere via their root nodules and 33% from the soil.

After the peas were harvested, the pots were sown with oats (Guldregn II, Svalöv), 10 seeds to each pot. The same watering solution was used, free from combined N, as in the experiment with the peas. The first oat crop (I) was followed by a second (II) using the same number of plants and the same nutrient supply. The results of these experiments are given in Table III.

TABLE III

After-effect of peas inoculated with bacterial strains H VIII and H 47 on the uptake of nitrogen by oats. Two successive oat crops taken after pea, 10 oats per pot. Period of growth: I oat crop: April 5–July 9, 1951. II oat crop: July 20–October–10 1951. Natural light.

No. of pot	Bacterial strain	I oat crop		II oat crop		I + II oat crops
		Dry weight, g	N mg	Dry weight, g	N mg	N mg
1	H VIII	12.501	200.0	3.355	39.3	239.3
2	H VIII	10.480	155.1	4.162	40.4	195.5
3	H VIII	16.100	204.5	3.778	33.6	238.1
5	H VIII	14.101	143.9	4.334	47.2	191.1
	Average	13.295	175.8	3.907	40.1	216.0
1	H 47	21.065	263.3	5.709	49.3	312.6
2	H 47	15.243	218.0	5.233	53.4	271.4
3	H 47	19.285	198.6	6.417	52.0	250.6
5	H 47	20.724	217.6	4.795	41.2	258.8
	Average	19.079	224.4	5.381	49.0	273.4

The two oat crops following the peas inoculated with an effective strain together contained an average of 57.5 mg more N per pot than the crops following the peas with an ineffective strain. For the first crop (I), the difference between the average values was 224.4–175.8 i.e. 48.6 mg for the second crop (II)49.0–40.1 i.e. 8.9 mg N per pot. In both series, the values for the second oat crop were low, in other words the after-effect of peas inoculated with an effective bacterial strain falls appreciably after the first oat cropping. Nevertheless, the effect is still detectable. The results obtained with the second oat crop, however, suggest that a third crop would have no longer benefited to any extent from peas inoculated with an effective strain.

Assuming that the increase in the N content of the oats following the peas inoculated with the effective strain (57.5 mg per pot) was due to N fixation and taking into account the difference between the N contents of the two pea crops themselves (105 mg per pot) then the total amount of N fixed in the nodules amounts to 162.5 mg per pot of 4 pea plants. Since the surface area of the pots was 254 cm^2 the amount of N fixed in a pea culture inoculated with an effective bacterial strain would be 65 kg per ha, of which about 65% would be found in the pea crop itself and the remainder in the roots or possibly

partly as excretion products in the soil. It is of course possible that part of the extra N in the oats following the peas with effective nodules may be due to changes affected by the peas in the mobilization of the nitrogen compounds originally in the soil. There is, however, no evidence for this.

After harvesting the peas, analyses of the soil were made for total N, soluble N and NO_3-N. For total N, two soil cores (extending from top to bottom) were taken from each pot, mixed and dried at 40°C — in all about 40 g of soil. Soluble N was determined by adding water to a 100 g sample of soil to give a total volume of 500 ml and shaking the suspension for 4 hours at room temperature. The N analyses were made on 10 ml of the filtrate. 25 ml of the same filtrate was used for the determination of NO_3-N. The solution was evaporated to dryness, phenyl sulphonic acid added and the intensity of the colour determined in alkaline solution by means of a Klett photometer. The analytical results are given in Table IV.

TABLE IV

Total N, soluble N, and NO_3-N in the soil after harvest of pea

No. of pot	Bacterial strain	Total N mg/kg soil	Soluble N mg/kg soil	NO_3-N mg/kg soil
2	H VIII	3220	60	1.4
3	H VIII	3157	60	1.1
4	H VIII	3087	75	1.2
5	H VIII	3200	75	1.3
	Average	3166	67	1.2
1	H 47	3147	65	4.3
3	H 47	3059	60	2.4
4	H 47	3203	65	1.7
5	H 47	3255	60	2.8
	Average	3166	63	2.8

The values for total N in the soil vary so much from pot to pot that no conclusions can be drawn as to changes following the pea cultures. This was to be expected in view of the fluctuations in the N content of the soil where the pea roots remained, although, by chance, both series gave the same average values.

The soluble N contents of the soils from both series show little difference but the NO_3-N content is somewhat higher in the series with effective nodules.

Whilst in these experiments the oats were grown in natural light during the spring and summer, the peas were grown in artificial light during the winter. Under these conditions pea growth has been about half that obtained in similar pot experiments during the summer. Nitrogen fixation under natural light conditions therefore might be expected to be over 100 kg per hectare. Since the aim of the experiment was to test the applicability of this new principle to the determination of the extent of nitrogen fixation in root nodules in the soil, the size of the crop is of no real significance.

This method is probably also applicable to field experiments. Since, however, the legume seedlings have to be grown so that only a desired bacterial strain has the chance to produce the first nodules on the roots, the raising of sufficiently large numbers of seedlings necessary for field experiments is very laborious. Nevertheless the method is still feasible when only small plots are used. Next summer we hope to arrange a corresponding experiment in the field.

In the experiments described above, the peas took up from the soil less combined nitrogen than did the oats. It is true that there were only four peas compared with 10 oats in each pot, so that the roots of the latter presented a much greater surface area. Even so, it would appear that oat roots are still more effective than pea roots in the uptake of nitrogen, as evidenced by pot experiments in which oats were grown at the same time as the peas and in the same kind of soil. Table V gives the results of an experiment in which 3 successive crops of oats were grown in two pots, each with 10 plants. The experiment was carried out at the same time as the experiments with pea, (oats I) and oats (II) described above.

TABLE V

Three successive oat crops, 10 oats per pot. No N-nutrition. Soil the same as in the pea series. Period of growth: I oat crop: Jan. 29–March 19, II oat crop: April 5–July 20, III oat crop: July 20–Oct. 10.

No. of pot	I crop		II crop		III crop	
	Dry matter g	N mg	Dry matter g	N mg	Dry matter g	N mg
1	14.454	232.7	8.880	101.2	5.131	49.3
2	18.393	209.7	9.689	139.5	4.569	42.0
Average	16.424	221.2	9.285	120.4	4.850	45.7

Allowing for the N content of the seeds (7 mg per 10 oat seeds, 28

mg per 4 pea seeds) the first crop of oats has taken up approximately 214 mg N whilst the peas inoculated with the ineffective strain H VIII have taken up only 44 mg. The second oat crop has taken up 113 mg N compared with 169 mg N taken up by the first oat crop following these peas. The third successive oat crop has taken up 39 mg N, the second oat crop following the peas 33 mg Overall, the 3 successive oat crops have together taken up 214 + 113 + 39, i.e. 366 mg N whilst the peas followed by 2 oat crops have taken up only 44 + 169 + 33, i.e. 246 mg N. The difference is surprisingly great and difficult to explain. Although the oat series was only replicated twice, the two replicates gave very similar results.

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SUMMARY

A method has been described for the determination of the rate of nitrogen fixation when peas are grown in a soil from which they can take up combined nitrogen. This method is based on an earlier observation that previous nodule formation by an ineffective strain of the pea *Rhizobium* H VIII prevents later nodulation by effective strains. Parallel pot experiments (muddy clay soil) in which pea plants were inoculated either with the ineffective strain VIII or with the effective strain H 47, showed that no effective nodules were produced by the former within a period of 2 months. Differences between the nitrogen contents of the peas in the two cultures can therefore be assumed to indicate the amount of atmospheric nitrogen fixed. Two successive oat crops were grown after the peas in order to determine the after-effect of the peas.

The oats took up from the soil much more nitrogen than did the peas.

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MOLYBDENUM NUTRITION OF CROP PLANTS

II. PLANT AND SOIL FACTORS CONCERNED WITH MOLYBDENUM DEFICIENCIES IN CROP PLANTS

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INTRODUCTION

The first paper of this series [13]) discussed the absorption of molybdenum by plants from culture solutions and soils as influenced by the sulfate and phosphate supplies and hydrogen ion concentrations of the nutrient media. This paper deals with the relative abilities of a number of different crop plants to grow on the same low molybdenum soil medium. The soil chosen for the experiments was sufficiently low in molybdenum to produce acute molybdenum deficiency symptoms in some of the species. However, in other species, no visual indications of molybdenum stress were observed under the same conditions of cropping.

Reasons for the development of molybdenum deficiency in serpentine soils were in themselves of considerable interest. Toward the end of verifying postulated chemical, geological, and weathering factors giving rise to low molybdenum supplying power of such soils, a limited survey of selected soils was made.

EXPERIMENTAL METHODS

Selection of Soil of Low Molybdenum Supplying Power

A convenient source of soil of low molybdenum supplying power was provided by California serpentine soils used by W a l k e r [15]) who observed molybdenum deficiency in tomato and lettuce plants grown on them. Because molybdenum stress was shown by tomato and lettuce, it was assumed that these particular soils were lower in available molybdenum than the Australian soils discussed by A n-

derson[1]) and Anderson and Thomas[3]). In Australia it had been repeatedly observed that molybdenum applications enhanced the power of legumes to fix nitrogen but did not benefit the growth of non-legumes.

Fertilization and Irrigation

Besides being low in molybdenum, the soil used in these experiments could be characterized as low in phosphorus, sulfur and calcium and high in magnesium.

High magnesium contents of soils developed from serpentine parent material effectively result in calcium deficiency of agricultural plants. However, if the exchangeable calcium was raised to 20 per cent of the exchange capacity, the calcium nutrition of tomato and lettuce plants was adequate [14]).

The following high calcium fertilizer (hereinafter referred to as the "high Ca" fertilizer) was formulated to provide a maximal calcium level for the high magnesium serpentine soil. It also provided nitrogen, phosphorus, sulfur and potassium in amounts such that these elements did not become limiting during the cropping period. The unit of the high calcium fertilizer which was added to each 100 grams of soil, contained 1.4 m.mols $Ca(NO_3)_2$, 0.288 m.mols $Ca(H_2PO_4)_2$ and 0.163 m.mols K_2SO_4. Sufficient Na_2BO_2 to make the soil 0.5 p.p.m. with respect to boron was also added.

The serpentine soil (hereinafter referred to as soil No. 177) had a cation exchange capacity of 27 milliequivalents per 100 g, of which only 11% was satisfied by calcium. Addition of the unit of the "high Ca" fertilizer increased the soil calcium level to 23.5% of the total soluble and exchangeable cations.

This rate of fertilizer application is high, but still not great enough to give rise to deleterious osmotic pressures in the soil solution. At the moisture equivalent of Soil No. 177 (37%) the osmotic pressure from a unit of the added fertilizer was approximately 3.5 atmosphere at the beginning of cropping. During cropping an attempt was made to keep the soil moisture levels at the moisture equivalent or above.

No provision was made for drainage of the culture pots. Thus uniform and adequate irrigation depended upon the judgment and skill of those attending the growing plants. Because the soil was visible through the glass containers, it was possible to make frequent irrigations and to avoid filling pore-spaces with water.

The method of fertilization and irrigation described above was found generally effective in making other non-saline infertile soils productive. For example, as will be discussed later, a great many different serpentine soils were tested in our greenhouses for molybdenum supplying power. When molybdenum was included, application of one unit of the high calcium fertilizer to each 100 grams of the soil always resulted in good growth of tomatoes.

Precautions to Prevent Molybdenum Contamination

Acid washed pyrex glass containers were used in all experiments. In the first soil experiments (fall 1948) 2 liter pyrex beakers were used to contain 2 kilograms of air dry soil. In later experiments 1500 grams of soil were placed in rectangular pyrex dishes of the following dimensions: length 25 cm, width 12 cm, depth 9 cm. No covers were provided.

All fertilizing salts except the Na_2BO_2 were purified by coprecipitation of MoS_3 with CuS as described by M e a g h e r [7]). Pyrex glass distilled water was used for irrigation.

Method of Cropping

The amounts of air dry soil required for each culture were weighed out and treated with the "high Ca" fertilizer dissolved in enough water to bring the soil to approximately half its moisture equivalent. After thoroughly mixing with a rolling cloth the soil was placed in containers. Further water was then added and seeds were planted. After seedlings were germinated they were thinned to 4 to 10 plants depending on species. Each culture was replicated four times.

Additions of molybdenum were purposely delayed until symptoms of molybdenum deficiency were recognized. For some species, deficiency symptoms were obvious and additional information was made available by observing recovery of the plants following molybdenum additions to the soil. For other plants whose symptomology was unknown, or which showed no clear-cut indications of abnormal development, a planned program of delayed molybdenum fertilization could not be followed.

Time of harvesting depended upon many factors, but in general the crops showing molybdenum deficiency symptoms were harvested before they had initiated fruiting. However, the beans, peas and cowpeas which did not show molybdenum deficiency symptoms were maintained in order to obtain seed.

Seasonal variations in climate have been shown to effect plant requirements for micronutrient elements such as manganese and zinc. In general, slow growing winter plants have lower physiological requirements than others grown in the spring and summer seasons. In these experiments the first crops were grown in the fall, and some of the species showed no recognizable signs of molybdenum stress. Consequently, all species not showing a response to molybdenum in the fall were again cropped in the spring. In addition, three of the crops (tomato, sugar beet and broccoli) showing acute molybdenum deficiency symptoms in the fall and winter months were cropped again in the spring for comparative purposes relating to seasonal response.

In order to amplify information as to the relative abilities of different species to absorb adequate amounts of molybdenum from a low molybdenum soil, altogether thirty different crop plants have been grown in soil No. 177 under the experimental conditions described.

Chemical Methods of Analysis

After harvesting, the plants were dried at 70°C in a forced draft drying oven. In order to prevent contamination, samples to be analyzed for molybdenum were hand crushed. The remainder of the sample was ground in a Wiley mill prior to analysis for nitrates.

Molybdenum was determined colorimetrically as molybdenum thiocyanate [10]. For the purpose of these investigations, several modifications of the general method have been made which have permitted spectrophotometric measurements of 1.0–10.0 micrograms of molybdenum with a precision of $\pm$ 5%, and 0.1–1.0 micrograms with a precision of $\pm$ 10%.

Nitrates were determined colorimetrically by the method of Johnson and Ulrich [6].

FALL EXPERIMENTS

Ten plant species, five legumes and five non-legumes were planted in two liter Pyrex beakers containing two kilograms of soil No. 177, fertilized with 20 units of the "high Ca" fertilizer. Four pots were planted to each species. Two were kept as controls and two received later molybdenum supplements, as will be described. The legumes were subterranean clover (*Trifolium subterraneum*), white Dutch clover (*Trifolium repens*), dwarf horticultural beans (*Phaseolus vulgaris*), cowpeas (*Vigna sinensis*) and garden peas (*Pisum sativum*)

and the non-legumes were tomatoes (*Lycopersicum esculentum*), broccoli (*Brassica oleracea var. botrytis*), buckwheat (*Fagopyrum esculentum*), squash (*Cucurbita pepo var. verrucosa*) and sugar beets (*Beta vulgaris var. crassa*, strain U.S. No. 15).

During the cropping period (Aug. 18, 1948 to October 10, 1948) no observable symptoms suggestive of molybdenum deficiency were observed for any of the legumes, but were evident in different degrees for each of the non-legumes. Molybdenum was added as Na_2MoO_4 at the rate of 0.67 lb Mo per acre (2 mg Mo per pot) four weeks after planting, at which time molybdenum deficiency symptoms were well recognized in the tomatoes, the only crop with whose molybdenum deficiency symptoms we were thoroughly familiar. However, remaining non-legumes also showed abnormalities which disappeared after molybdenum was added. Sugar beets in particular were very pale and also had started to die back from the tips of the blades. Nearly all the blades of the sugar beets tended to curl upward from the midrib, but the characteristic interveinal mottling shown on tomatoes was not evident. Broccoli was severely injured, having in addition to pale, curled blades similar to that shown for the sugar beets, a pronounced necrosis beginning in the interveinal areas. Squash had pale leaves and a characteristic interveinal motling. Buckwheat was the least affected, showing only a slight interveinal mottling and a tendency in some of the blades to assume a cupped form. After molybdenum was added to the soil, observations were made every 24 hours, comparing the treated cultures with the controls. After the first 24 hours greening up was observed in tomato, broccoli and sugar beets and the rate of progressive dieback of the sugar beet blades was diminished. After 48 hours, recovery was pronounced for these three species. After 96 hours the treated tomatoes showed complete recovery, whereas the untreated ones had continued to decline. Broccoli also showed complete greening up except for the necrotic areas, which were sharply delimited, having healthy green tissue immediately adjacent to dead leaf tissue at positions where progressive necrosis of interveinal areas was arrested upon addition of molybdenum.

Squash and buckwheat were not as severely affected at the time of molybdenum addition and, therefore, differences between plants treated with molybdenum and those not treated were not as great. The early assumption that the pale leaf color and the tendency

toward blade cupping was associated with incipient molybdenum deficiency was strengthened since the molybdenum treatment resulted in complete disappearance of blade cupping, whereas the plants not receiving molybdenum had about 20 percent of their blades so affected. Moreover, with increasing age of the untreated plants, the cupped blades gradually became paler with some of them dying.

Further remarks on the rates of recovery of treated molybdenum deficient plants are in order because of the ease with which treatments may be used as a supplement to the diagnosis of molybdenum deficiency. Injured broccoli and sugar beet plants particularly showed spectacular responses to molybdenum additions as described below. On Oct. 6, 1948, 53 days after planting, 0.05 ml of a solution containing 0.05 mg Mo was placed in a leaf bract of one of the deficient broccoli plants. At this stage of growth, new leaves showed necrosis within three to four days after emerging. The outer edges and also some interveinal areas were pale and had a water-soaked appearance. The fleshy portions of the blades which had not yet become watery greened up on the second day after treatment and remained as green fleshy tissue. Thus the response to molybdenum was observed as the prompt arrest of progressive necrosis and the restoration of tissues not too severely injured

Sugar beets respond to leaf applications of molybdenum in manner similar to that shown by broccoli, for example, one of a pair of sugar beet plants grown in one pot was treated by placing 0.5 ml of a solution containing 0.5 mg of molybdenum at the base of the petioles near the root crown. Recovery was not as prompt as with the broccoli plant, but within four days greening up had started and in seven days all the treated plant leaves were a deep green. Also the treated plant attained a size about 50% greater than the untreated plant growing in the same pot.

Relative Abilities of Species to Satisfy their Molybdenum Requirements from a Given Soil Medium

From these experiments it was clear that different plant species varied greatly in their ability to acquire adequate supplies of molybdenum to meet their growth requirements. Within the limited numbers of species in the experiment, legumes thrived in the same soil that gave rise to acute molybdenum deficiency symptoms in tomato and sugar beet for example.

Intercropping of Beets and Clover. In order to extend the above observations, an additional experiment was devised in which sugar beets were interplanted with white Dutch clover and also with subterranean clover in soil No 177, with and without the addition of molybdenum. For this experiment the pots formerly planted to white Dutch clover and subterranean clover were reserved for the planting of sugar beets as follows: Half of the clover pots of the Fall experiment had the tops harvested, and the soil was allowed to dry with the clover roots remaining. The other half of the clover pots was allowed to remain unharvested. Sugar beets were then planted in all of the pots. Soon after germination, the sugar beets became acutely molybdenum deficient in the pots which had received no molybdenum supplement. Normal growth of sugar beets took place in pots which had been fertilized with molybdenum. Beets growing intermixed with the clover showed the same kinds of growth as beets grown following the clover crops, and as beets which were grown on the original soil. It was interesting to observe acute molybdenum deficiency of sugar beets growing in the same pots with clover showing no evidence of molybdenum stress.

In order to explain the enormously different molybdenum responses observed for the several plant species, it was necessary to assume differences in one of the following: the ability of plants to extract molybdenum from the soil, differences in the amount of molybdenum contamination carried by the seed, or differences in the amounts of molybdenum required by the plant *per se*.

In connection with the latter, there still remained the possibility that some plant species might not require molybdenum. At the time, reference to the literature failed to reveal any reports that molybdenum was essential for the growth of legumes. Consequently, experiments were initiated in which Dwarf horticultural beans and peas were grown in culture solutions for the purpose of determining whether molybdenum was essential for their growth. Results from the culture solution experiments showed molybdenum to be essential for the growth of both of these legumes [8] *).

*) Since this experimental work was completed, Evans, Purvis and Bear have published their paper [4]) showing that alfalfa requires molybdenum for growth. Hewitt [5]) also has reported that acute molybdenum deficiency symptoms have been observed on lucerne and clover when supplied with fixed nitrogen. Wilson [16]) has diagnosed the scald disease of beans as a molybdenum deficiency of that plant.

SPRING EXPERIMENTS

The great differences in response to molybdenum fertilization observed for the ten species studied in the fall of 1948 led us to initiate additional experiments using a larger number of agriculturally important plants. Included in Table I are the species grown in the fall of 1948 and spring of 1949.

Cultural techniques in the spring experiments were the same as those previously described except that the pots were rectangular Pyrex dishes, 25 cm long, 12 cm wide and 9 cm deep. They contained 1500 grams of soil 177 and were fertilized with 15 units of the "high Ca" fertilizer. Two pots for each kind of plant received no molybdenum. Two other pots were treated, each with two milligrams of molybdenum, by surface application of a solution of Na_2MoO_4, prior to planting the seeds. Seeds were planted in the soil on March 4, 1949, and the seedlings were thinned to four plants per pot as soon as the plants became established. The first new leaves of broccoli, kale, cabbage, spinach, squash and chard were chlorotic in both the plus and minus molybdenum treatments. It was thought that these plants might have had a higher molybdenum requirement than the other species under investigation. Therefore, on April 2, 1949, an additional 4 milligrams of molybdenum was added to each of the plus molybdenum pots for each of these crops.

These extra supplements of molybdenum are taken into account in the discussion pertaining to the molybdenum contents of these plants on page 191. Later developments have indicated that the early chlorosis of these seedlings plants was distinct from that caused by molybdenum deficiency, and was due to some other factor as yet undetermined.

Three weeks after planting, sugar beets exhibited molybdenum deficiency symptoms very clearly; but turnips, mustard, kale, cabbage, broccoli and chard showed symptoms which were only suggestive of molybdenum deficiency. Five weeks after planting, sugar beets and table beets showed very marked molybdenum deficiency on all untreated plants and none on treated pots. Table beets showed symptoms of leaf curl and tip die back very similar to sugar beets, differing only in that the deep red coloring in the veins of the table beet leaves became most conspicuous on the yellow chlorotic background. Turnips, radishes, chard, spinach, cabbage, kale, broccoli,

lettuce, tobacco, and tomato all showed symptoms in varying degree. None of the grasses *), barley, wheat, oats, maize or sorghum, showed any evidence of molybdenum deficiency symptoms or of depressed growth when compared with their molybdenum-treated counterparts.

As the experiment progressed, it became obvious that age of the plant modified the gross aspects of response to molybdenum treatment. For example, some species appeared to thrive without added molybdenum for some time, then, having exhausted the reserve of easily available molybdenum, either from the seed or from the soil, became acutely deficient. Other species, most notably lettuce and tobacco, grew extremely slowly for the first two or three weeks when not receiving molybdenum supplements, but then began to make fair growth, however not approaching that of the plants in the pots receiving added molybdenum. Others, such as lettuce, remained alive but failed to grow beyond a few centimeters in height unless supplemented with molybdenum.

CHARACTERIZATION OF DEGREE OF INJURY SHOWN BY DIFFERENT PLANT SPECIES GROWN IN SOIL NO. 177

At the termination of the spring experiments (April 29, 1949) the different plants used in both fall and spring experiments were classified in accord with the severity of symptoms associated with molybdenum deficiency.

Group 0 included those plant species which showed no evidence of molybdenum deficiency when grown in soil No. 177 without supplemental molybdenum and no increase in yield or change in foliage appearance when molybdenum was added to the soil. All the legumes and grasses studied fell in this group (Table I). All other plants showed varying degrees of foliar symptoms of molybdenum deficiency.

Buckwheat and radish were placed in group 1. These plants were pale in appearance, slightly necrotic and showed leaf cupping in buckwheat, but growth was not restricted in the control when compared with that obtained in the plus molybdenum treatment. Group 2 included carrots, broccoli, kale, squash and parsnips. The growth

*) In experiments now in progress, Timothy (*Phleum pratense*) exhibits definite chlorosis but no reduction of growth when grown without supplemental molybdenum.

TABLE I

Effect of Molybdenum Fertilization on Molybdenum and Nitrate Concentrations in Plants Grown in a Molybdenum Deficient Soil
(2 mg Mo per pot added in all plus molybdenum treatments except as noted)

	Response Group	Season	Mo in tops p.p.m. Dry wt.		Nitrate in tops % Dry weight	
			+ Mo	noMo.	+ Mo	noMo
Leguminosae						
Beans, *Phaseolus vulgaris*	0	F	5.2	0.40	—	—
Cowpeas, *Vigna sinensis*	0	F	4.7	0.20	—	—
Garden Peas, *Pisum sativum*	0	F	3.2	0.34	—	—
Clover, *Trifolium subterraneum*	0	F	3.1	0.15	—	—
Clover, *Trifolium repens*	0	F	4.0	0.12	0.40	0.93
Clover, *Melilotus alba*	0	S *)	0.93	0.04	—	—
Trefoil, *Lotus corniculatus*	0	S *)	0.75	0.42	—	—
Graminae						
Barley, *Hordeum vulgare*	0	S	3.07	0.03	1.06	1.96
Maize, *Zea Mays*	0	S	1.03	0.07	0.24	0.30
Oats, *Avena sativa*	0	S	0.55	0.03	0.67	0.88
Sorghum, *Sorghum vulgare*	0	S	0.48	0.11	1.20	0.93
Wheat, *Triticum aestivum*	0	S	0.55	0.03	2.44	4.70
Chenopodiaceae						
†) Chard, *Beta vulgaris* var. *cicla*	3	S	8.94	0.10	2.52	8.72
Table Beet, *Beta vulgaris* var. *crassa*	4	S	0.62	0.05	1.86	14.70
Sugar Beet, ,, ,, ,, ,,	4	S	0.72	0.13	2.08	14.05
†) Spinach, *Spinacia oleracea* var. *crassa*	5	S	1.61	0.10	0.44	10.40
Compositae						
Lettuce, *Lactuca sativa* var. *capitata*	4	S	0.09	—	2.88	11.30
Cruciferae						
†) Broccoli, *Brassica oleracea* var. *botrytis*	2	S	16.2	0.04	2.17	11.30
†) Cabbage, *Brassica oleracea* var. *capitata*	4	S	25.9	0.05	4.87	15.20
†) Kale, *Brassica oleracea* var. *acephela*	2	S	17.1	0.06	4.87	8.40
Mustard, *Brassica campestris*	3	S	1.4	0.01	3.14	9.30
Radish, *Raphanus sativus*	1	S	2.43	0.06	—	—
Turnips, *Brassica rapa*	4	S	1.16	0.03	3.19	11.70
Solanaceae						
Potato, *Solanum tuberosum*	3	S	8.80	0.07	4.65	10.85
Tobacco, *Nicotiana tabacum*	3	S	1.08	—	3.32	17.03
Tomato, *Lycopersicum esculentum*	3	F	0.68	0.13	0.95	9.88
,, ,, ,,	3	S	0.61	0.13	—	—
Polygonaceae						
Buckwheat, *Fagopyrum esculentum*	1	F	0.72	0.11	—	—
Cucurbitaceae						
†) Squash, *Cucurbita pepo* var. *verrucosa*	2	F	5.0	0.20	0.31	6.12
Umbelliferae						
Carrots, *Daucus carota*	2	S	0.45	0.05	3.10	10.30
Parsnips, *Pastinaca sativa*	2	S	1.82	0.04	0.63	2.03

*) Second crops following buckwheat and squash.
†) Received 6 mg Mo per pot.

of these plants in the untreated pots was restricted, the blades were chlorotic. Also necrotic areas were prominent.

Restricted growth, severe chlorosis and the death of many complete leaves characterized the plants of group 3. Tomato, tobacco, potato, mustard and chard were included in group 3. The plants in group 4, sugar beets, table beets, turnips, cabbage and lettuce made extremely poor growth in the minus molybdenum pots. The leaves were chlorotic to blanched and older leaves died before becoming very large.

Group 5 was reserved for plants which, without receiving supplementary molybdenum during the time of the experiment (8 weeks) had their leaves and all other plant parts die. Only spinach came in this group.

SIGNIFICANCE OF MOLYBDENUM CONCENTRATIONS IN PLANTS IN RELATION TO THEIR GROWTH

It should be emphasized that the above method of characterizing growth responses is highly subjective and can be regarded only as a qualitative indication of the relative ability of these crop plants to thrive on the molybdenum deficient soil. The most significant information arising from these experiments is that the members of the families *Leguminosae* and *Graminae* so far tested have relatively low requirements for molybdenum as indicated by plants of these families making normal growth on the soil without molybdenum supplements and exhibiting no foliar symptoms of molybdenum deficiency. Addition of molybdenum to the soil did not result in enhanced growth of members of these families although increases in molybdenum concentration in the foliage were noted in all cases (see Table I). Since the legumes and grasses (supplied with fixed nitrogen) did not exhibit molybdenum deficiency symptoms the following questions were raised with regard to them as contrasted with the other plants. (1) Did their seeds have enough molybdenum to allow them to develop normally? (2) Was their physiological requirement for molybdenum exceptionally low? (3) Did these plants extract soil molybdenum more efficiently than other plants showing molybdenum deficiency symptoms? Analysis of seeds revealed that the cowpeas, garden peas and beans contained large amounts of molybdenum — 0.72, 1.40 and 4.33 micrograms per seed respectively. Also, the molybdenum contents of different lots of seed were found

to vary considerably. For example, of two lots of bean seed examined, one lot contained 4.33 microgram per seed but another contained 1.00 microgram per seed. The other legumes, subterranean clover, white clover, and trefoil contained 0.016, 0.001 and 0.0025 micrograms molybdenum per seed respectively. Thus it could be expected that seed borne molybdenum would be of less importance in the nutrition of the clovers than for cowpeas or beans. The molybdenum content of other seeds used in these experiments are given in Table II.

TABLE II

Molybdenum Contents of Various Seeds (air dry basis)

	Micro grams per gram	Average wt of Mo. per seed (micrograms)/seed
Beet-Garden	0.143	0.0022
Beet-Sugar	0.100	0.001
Chard	0.231	0.003
Spinach	0.131	0.001
Lettuce	0.258	0.0002
Broccoli	0.124	0.0006
Cabbage	0.341	0.0011
Kale	0.112	0.0004
Mustard	0.343	0.0012
Radish	0.430	0.0037
Turnip	0.236	0.0004
Squash Lot 1	0.762	0.0587
Lot 2	0.980	0.0825
Barley	0.296	0.0141
Maize	0.564	0.136
Oats	0.764	0.0240
Sorghum	1.37	0.0168
Wheat Lot 1	0.280	0.0098
Lot 2	0.382	0.0149
Buckwheat	0.459	0.0114
Tobacco	0.528	0.000005
Tomato Lot 1	1.462	0.0051
Lot 2	1.59	0.0053
Carrot	3.72	0.0093
Parsnip	1.09	0.0052
Beans — Dwarf Lot 1	2.32	1.000
Lot 2	8.29	4.330
Clover — White Dutch	1.67	0.001
Clover — Subterranean	2.77	0.016
Cowpeas	2.61	0.718
Peas — Garden	3.72	1.396
Trefoil-Birdsfoot	2.61	0.0025

Certain limited conclusions can be made with respect to the above questions from the data of Table I and also Table III which has been

compiled to show the amount of molybdenum removed by the foliage of the crops relative to seed borne molybdenum. In Table III it is shown that the seed of cowpeas for example could have contributed most of the molybdenum found in the crop at time of harvest. In the case of barley, oats and wheat, nearly half could have come from the seed. However, a large proportion of the molybdenum found in the sweet corn and sorghum, and almost all of that found in white Dutch clover, sugar beet, table beet and tomato had to be derived from the soil rather than from the seed. Since the relative contribution of seed borne molybdenum varied considerably within the legumes as with the grasses, the molybdenum contents of their seeds could not completely explain their apparent ability to thrive without molybdenum supplements.

TABLE III

Comparison of Seed Borne Molybdenum with Molybdenum Removed by Foliage of the Plants after Cropping 8 Weeks

	No. of plants	Yield	p.p.m. Mo	μ g removed by crop	μ g added by seed	μ g Mo in crop less seed Mo
Cowpeas	4	17.6	.20	3.52	2.88	.64
White Dutch Clover	10	7.4	.12	.84	.01	.83
Sugar Beets	9	2.9	.13	.38	.009	.37
Table Beets	4	1.5	.05	.08	.008	.07
Tomato	6	6.72	.13	.87	.03	.87
Barley	10	14.0	.03	.42	.14	.28
Sweet Corn	4	41.0	.07	2.97	5.44	2.43
Oats	10	13.8	.03	.41	.24	.17
Sorghum	4	11.2	.11	1.23	.07	1.16
Wheat	10	7.0	.03	.21	.10	.11

The second question regarding the physiologically required concentrations of molybdenum would indicate that white Dutch clover, sweet corn and sorghum might require lower concentrations of molybdenum than sugar beets or tomato from the following reasoning. Since the former healthy species had the same or lower molybdenum concentrations than severely inhibited tomatoes or sugar beets, it could be inferred that they required less molybdenum for healthy growth. Similar but more convincing evidence is found in the even lower molybdenum contents of healthy oats, wheat, and barley. These plants made normal growth with 0.03 parts per million of molybdenum as compared to the molybdenum deficient sugar beets and tomatoes containing 0.13 parts per million. However, it must be

recognized that such comparisons between species are complicated by many factors perhaps including incompatability of concentration values as indicators of the nutrient status of plants under nutritional stress as shown by Steenbjerg [11]).

As for the efficiency with which different plants extracted molybdenum from soil No. 177, it may be stated categorically that the crop of sweet corn which showed no indication of molybdenum deficiency removed 2.4 micrograms of molybdenum from a given volume of soil whereas the more sensitive tomato and sugar beet plants removed 0.84 and 0.37 micrograms of molybdenum respectively. Other figures in column 7 of Table III indicate great differences in the abilities of different plant species to remove molybdenum from a given volume of soil having a low molybdenum supplying power.

It is also of interest that plants supplied with the same amount of molybdenum, accumulated it to quite different degrees. For example six milligrams of molybdenum were supplied to the plus molybdenum treatments for chard, spinach, broccoli, cabbage, kale and squash for reasons earlier stated (p. 185). The respective concentrations of molybdenum found at the time of harvest were 8.9, 1.6, 16.2, 25.9, 17.1 and 5.0 p.p.m. molybdenum respectively (Table I). The other species received 2.0 mg molybdenum per pot and at the time of harvest, were found to have molybdenum concentrations ranging between 0.09 p.p.m. for lettuce and 8.8. p.p.m. for potato.

The power of different plant species to accumulate molybdenum from high or low levels of supply is highly variable and depends on the particular plant in question. Thus broad generalizations regarding the significance of molybdenum concentrations in plants as related to their growth are not warranted.

GENERAL REMARKS ON FOLIAR SYMPTOMS OF MOLYBDENUM DEFICIENCY

The most common visual manifestation of molybdenum deficiency is leaf chlorosis. The chlorosis may exhibit an interveinal pattern in some plants for example, tomato, squash, broccoli, kale and cabbage. With other species of plants observed in the group discussed here, the chlorosis was of a more general and diffuse nature. For example, with the sugar beet the chlorosis was very similar to that associated with nitrogen deficiency.

Frequently a blade distortion is observed which may be referred to

as "cupping". It is of interest to speculate on the reasons for the characteristic blade cupping or blade curl that arises in plants suffering molybdenum stress. Abnormal blade shapes can be explained by assuming that molybdenum deficiency results in a diminished rate of blade expansion near the edges. The midribs and veins, however, remain relatively unaffected. Depending upon the geometric configuration of the blades of particular kinds of plants, the mechanical stresses involved by differential expansion rates results in either blade curling as in tomatoes and beets, or blade cupping of buckwheat and broccoli. Usually the cupping results from the upper blade surface being depressed at the midrib, but with some plants such as broccoli, cabbage, and kale, the blade portions along the midrib may be either depressed or raised depending upon the fortuitous circumstance of the surface geometry of the blade at the time when differential expansion rates become a dominant factor in determining future blade distortion. Broccoli and cabbage may have individual blades showing both types of distortion, resulting in an undulating pattern of the midrib (whiptail disease).

The idea that the growth rate of blade parts adjacent to the conducting elements is less limited than that of the remainder of the blade is compatible with the observation that blade tissue within a millimeter or so from the midribs and veins, remains green after death of the cells in the interveinal regions has occurred. Thus visual molybdenum deficiency symptoms consist of a general pattern of chlorosis, frequently associated with blade distortion, and under conditions of more severe deficiency, necrosis of interveinal tissues.

DISTRIBUTION OF NITRATE NITROGEN IN MOLYBDENUM DEFICIENT LEAVES

As further evidence of reduced physiological activity, and presumably growth, in the interveinal leaf areas; it has been found by Wilson and Waring[17]) that nitrate nitrogen is not reduced in these regions in molybdenum deficient cauliflower plants. The writers have also found that diphenylamine oxidizing substances presumably nitrates, were present only in traces in the interveinal areas of normal sugar beet blades but were present in large amounts in molybdenum deficient blades. The concentrations of nitrates in or near the midribs and veins of both normal and deficient blades were approximately the same. Chemical analyses of conducting element

tissues and blade tissue of sugar beets and tomatoes by the phenoldisulfonic acid method likewise showed that of molybdenum deficient blade tissue contained very high concentrations of nitrates as compared to petioles. Nitrate nitrogen of the blade was much reduced by the application of molybdenum to the cultures (Table I). The concentrations of nitrate in the conducting tissues were effected only slightly by changes in the molybdenum status of the plant.

DEVELOPMENT OF MOLYBDENUM DEFICIENCIES ON SERPENTINE SOILS

Following Walker's [15] discovery of a serpentine soil of low molybdenum supplying power, effort was directed toward surveying a limited number of soils developed from serpentine parent material with the expectation that they might also be low in molybdenum. The reasoning for this expectation was that the interaction of topographic and chemical factors operating during weathering of the serpentine rock should be favorable for the removal of molybdenum.

Topographic Factors

The primary serpentine soils under consideration here are present as thin mantles on ridges or moderate slopes, occupying high points in the general topography of the region probably as a result of the intrusive nature of serpentine formations. Thus they are well drained and it may be expected that they would be low in soluble ions, including molybdate ions. For example, as illustrated in the schematic diagram of Figure 1, soil *A* occupying the high, well

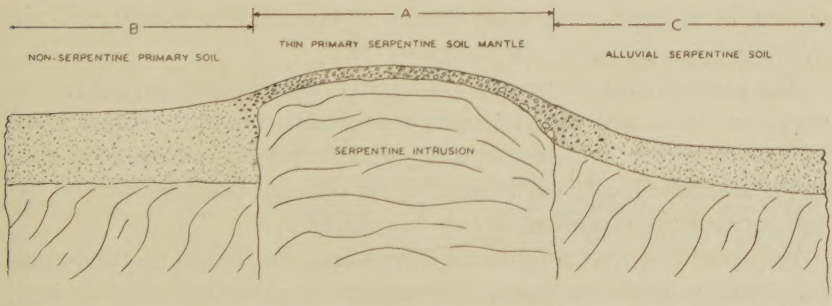

Fig. 1. Topographic features favorable to the development of serpentine soils of low molybdenum supplying power.

drained position would be lower in molybdenum than soil *B*, a non-serpentine primary soil or soil *C*, an alluvial soil of serpentine origin.

The latter soils would be expected to be higher in molybdenum because they receive the drainage waters from soil *A* and are also subject to more restricted drainage.

Chemical Factors

The topographic factors discussed above are of course pertinent only for non adsorbed ions or ions that may be released from the absorbed state in the course of hydrolyses by leaching waters. The chemical status giving rise to loss by leaching of anions such as molybdate, phosphate and sulfate is influenced by the high degree of cation saturation of the serpentine soil.

Serpentine barrens have only a sparse plant cover as compared to ajacent soils. Thus, it might be suggested that the high base status of serpentine soils is primarily a result of weathering by straight forward water solubility of parent rock with the soil water being little enhanced by carbonic acid derived through biological activities. The near neutral and sometimes slightly alkaline reactions characteristic of the serpentine soils, would prevent fixation of molybdates by anion adsorption processes and tend to assist removal of soluble molybdates with the leaching waters. The low molybdenum contents of the clay materials (less than 1 p.p.m. by fusion analysis (are in sharp contrast to the ironstone soils of Australia which have been reported by Oertel and Prescott [9]) to contain as much as 60 p.p.m. molybdenum. In the Australian situation ironstone soils having soil adsorbed molybdenum of 10 p.p.m. were made to supply molybdenum to plants by liming [2]). The assumed mechanism of release of molybdenum upon liming is exchange of molybdate for hydroxyl ions by anion exchange [12]) [13]).

The same process in neutral to slightly alkaline soils giving rise to very low total soil molybdenum would also tend to reduce their ability to retain phosphate and sulfate. Indeed, phosphorus and sulfur are also recognized as limiting for optimal plant growth on the serpentine soils examined in our laboratories.

Expectations that serpentine soils would be low in molybdenum supplying power were borne out in tests on fourteen soils secured from different sites and geographical locations.

SUMMARY

In order to provide a low molybdenum medium for comparative growth studies of a wide number of crop plants, a serpentine soil was treated with a

high calcium fertilizer to provide an adequate level of calcium as well as nitrogen, phosphorus, sulfur and potassium. Greenhouse studies of a number of different species all grown under the same cultural conditions revealed several different factors concerned with molybdenum deficiencies in crop plants.

Classification of the growth responses of the 30 different crop plants on the same low molybdenum soil permits certain tentative generalizations regarding the molybdenum extracting abilities and physiological requirements of the species studied under these experimental conditions. Of the 30 species studied, none of the legumes (seven in number) or grasses (five in number) required molybdenum, although a later experiment showed a need for molybdenum by timothy (*Phleum pratense*). The remaining 18 species all required molybdenum.

Consideration of the analytical data for molybdenum concentrations in seeds and the foliage of plants grown on this soil shows that seedborne molybdenum could account in part for the ability of some of the legumes (cowpeas and beans) and grasses (barley, oats and wheat) to grow normally on this medium, but that some of the species in these families (the clovers, maize and sorghum) must have been relatively efficient in extracting molybdenum from this soil because the seedborne molybdenum was entirely negligible as compared to the total amounts found in the foliage of mature plants.

Comparisons of the molybdenum concentrations in the foliage of plants grown on soil without molybdenum supplements suggest that some of the species may require higher concentrations of foliar molybdenum than do others. For example, healthy barley, maize, oats and wheat contained in the order of 0.03 to 0.07 p.p.m. molybdenum while tomatoes, sugar beet, squash, and spinach contained as much as 0.10–0.20 p.p.m. molybdenum while being moderately to actually molybdenum deficient, as judged from foliar symptoms and nitrate nitrogen concentration in the foliage.

The legumes and grasses studied evidenced no visible foliar symptoms of molybdenum deficiency when grown on the soil without supplemental molybdenum. Addition of 1 p.p.m. molybdenum (on dry soil basis) did not result in enhanced growth of these species although there was an increase in the concentration of molybdenum in the foliage.

The remaining 18 species showed varying degrees of foliar deficiency symptoms when grown on the soil without supplementary molybdenum. Addition of 1 p.p.m. molybdenum prevented the appearance of the foliar symptoms, resulting in increased growth and decreased nitrate nitrogen concentrations in the leaf tissue.

The most general foliar manifestation of molybdenum deficiency was leaf chlorosis. Deficiency in tomato, squash, broccoli, kale and cabbage was characterized by an interveinal chlorosis pattern but in many other species the chlorosis was more diffuse, resembling nitrogen deficiency. Blade distortion was frequently associated with molybdenum deficiency, as for example in the whiptail disease of cauliflower, cupping and curling of tomato blades, and cupping of sugar beet and buckwheat blades.

Chemical, geological and weathering factors in relation to the development of the low available molybdenum of serpentine soils are discussed.

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